MOSAICISM AND MUTATION IN *HABROBRACON*.

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In a recent publication (Whiting and Whiting, 1927) the hypothesis was advanced that certain irregular types in the parasitic wasp, *Habrobracon,* were caused by incomplete maturation of the egg. Impaternate females were assumed to originate from eggs in which there was failure of second maturation division. Mosaic males from unfertilized eggs of heterozygous mothers were thought to be due to failure of extrusion of second polar body, two oötids with different genes taking part in maturation; gynandromorphs to have originated in a manner similar to that of the mosaic males, except that one oötid was fertilized. A single heterozygous mosaic female was explained by mitotic irregularity in somatogenesis.

In the present paper are presented data giving additional evidence for the hypotheses previously advanced although no more impaternate females have been found. Factors considered here are the allelomorphs black, O, light, o^l, orange, o, and ivory, o^i (eye color), (Whiting and Burton, 1926) and normal, R, and reduced, r, (wings), (Whiting, 1926). Materials involved are stock 11, (type) from Iowa City, Iowa, which when previously used in crosses with Lancaster material gave the irregular types already discussed; stock 17 (ivory) and stock 20 (ivory reduced), both of mixed origin from the Lancaster and Iowa City materials; stock 24 (type) from Lowell, Massachusetts; and orange-eyed males from various stocks.

Stock 24 has not been previously described. A female, captured September 7, 1926 in Lowell, produced 24 males and 73 females. Thirteen virgin females produced 651 males. Thirty-

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TABLE I.

PROGENY FROM $Oo^{i}Rr$ FEMALES, VIRGIN OR CROSSED WITH VARIOUS MALES.

Parents.			Regular Offspring.								Freaks.		
F_1 ♀ ♀ $Oo^{i}Rr$.		♂♂.	♂♂.				♀♀.				Biparental ♂♂.	Mosaic and Mutant ♂♂.	♂♀.
No.	Source ♀ ♂		Type.	Ivory.	Reduced.	Ivory Reduced.	Type.	Orange.	Reduced.	Orange Reduced.			
24	20 × 11		994	989	940	934						283, 289, 319	
14	11 × 20		468	494	417	449						286, 287	
9	11 × 20	Orange	64	73	62	54	322	296			292	305	303
7	11 × 20	Orange Reduced	81	76	60	60	141	98	114	128	290, 291, 294		296, 302
6 6	20 × 24 24 × 20		308	315	277	298							
25 12	20 × 24 24 × 20	Orange	430	465	414	415	1,207	1,201			293?, 297, 298	306	
3 6	20 × 24 24 × 20	Orange Reduced	126	123	107	98	96	104	55	70	293a?, 295		
3	20 × 11	Ivory Reduced	55	52	36	46	100	Ivory 109	94	Ivory 89			
Total regular showing segregation.....			2,526	2,587	2,313	2,354	337	311	263	287			

six mated females produced 1417 males and 1129 females. These wasps were of normal appearance, like Lancaster "type" stock 1, but mesosternum was very "sooty" and 9 males and 6 females had breaks in wing vein r_4.

Reciprocal crosses were made between stocks 20 and 11 and between stocks 20 and 24. The F_1 females were in some cases isolated as virgin, in others bred to orange males (stocks 3, 12, 13) or orange reduced males (stock 19). Results in the segregating generation (from females Oo^iRr virgin or mated to oR or to or males) are given in Table 1 in which are also included three fraternities from F_1 females which had mated to their ivory reduced, o^ir, brothers.

Disregarding for the moment the 21 freaks which are designated by numbers at the right of the table, it may be seen that offspring fall into the expected classes on basis of independent segregation of ivory and reduced and dominance of orange over ivory. Males segregate in all cases while females segregate only when their fathers are recessive. None of the regular males are orange while ivory is concealed in all females except those with ivory fathers. Females segregating into the four classes are summarized as are all regular males at bottom of table. Reduced fall below expectation both in males, 47.7 per cent., and in females, 45.9 per cent. Recombinations have taken place in 50.1 per cent. of males and in 47.9 per cent. of females segregating for both factors. There is thus no evidence of linkage. In order to determine whether there might be linkage at some period during the mother's life or some effect of age of mother upon recombination of factors, segregating progenies of all cultures were added according to vials through which mothers were passed. No significant difference from average was found.

The infrequent types, "freaks," may now be considered.

Biparental Males.

Biparental males, formerly called anomalous or, because they are to be distinguished from other males by possessing paternal traits, patroclinous males, resemble their sisters in showing dominant characters inherited from either or both parents (Whiting, Anna R., in press). They are almost or quite sterile. Their few

daughters tend toward morphological abnormality and are usually sterile.

The nine males classed as biparentals in Table I are distinguished from the regular males by possessing orange eyes of paternal origin. They occur only in bisexual fraternities and only from vials containing females, in other words, before their mother's supply of sperm has become exhausted. Freaks 293 and 293*a* were not tested but are probably biparental. The remaining seven were almost or quite sterile. The four with long-winged father had long wings as expected. Of the five with reduced father, only one (290) had long wings received from its Rr mother.

Mutant and Mosaic Males.

Mutant Male 289.—An F_1 female from ivory reduced female by type male produced in addition to males,—type 75, ivory 61, reduced 69 and ivory reduced 60, an orange reduced male (289) (in vial *f*) with compound eyes rather dark and ocelli of typical orange color. He was mated with eight different females over a period of forty-four days producing daughters by each of them. A subsequent mating after ten days resulted in males only (58) indicating sterility due to age. Of the eight females producing daughters, two, orange reduced, produced 60 orange reduced daughters; five, ivory reduced, produced 172 orange reduced daughters, and one, type carrying reduced, produced 13 type and 4 reduced daughters. The eight mothers also produced 160 normal matroclinous males. Nine of the orange reduced daughters with orange reduced mothers were tested and produced only orange reduced offspring. Freak 289 therefore bred as he appeared somatically transmitting reduced in at least 236 cases and non-black (orange or ivory) in at least 232 cases of which at least 181 were certainly orange.

Mutant Male 319.—An F_1 female from ivory reduced female by type male produced in addition to males,—type 60, ivory 75, reduced 64 and ivory reduced 92, an orange reduced male (319) (in vial *g*) with compound eyes rather dark and ocelli typical for orange. He was mated over a period of fourteen days to eleven ivory reduced females. There were produced 490 ivory reduced males, 2 orange reduced (biparental) males, and 640 orange re-

duced females. Two subsequent matings resulted in males only, 207. Nine orange reduced daughters produced 218 orange reduced males and 210 ivory reduced males. No new types appeared.

Mutant Male 308.—Freak 305 (see below) had eyes which appeared mosaic for black and orange, gonads mosaic for black and ivory. All of his offspring had long wings. Four of his type (di-heterozygous $Oo^{i}Rr$) daughters from an ivory reduced mother (two had mated to ivory reduced brothers) produced males—type 129, ivory 139, reduced 134, and ivory reduced 121 and females—type 37, ivory 29, reduced 20, and ivory reduced 19. In one of these four fraternities which included females in vials *a, b,* and *c,* but males only in vial *d* there occurred, in vial *d*, an orange (deep red) long male (Freak 308).

Freak 308 was tested by mating to twelve ivory reduced females over a period of fifteen days. There were produced males—ivory reduced 701, orange long (biparental) 13, and females orange long 244. Two of these orange biparental males were tested by mating, twice each at an interval of eight days. Males of maternal type only appeared, 67 and 104 respectively, showing that biparental males from Freak 308 tend like other biparental males to be sterile.

Seven orange virgin daughters of Freak 308 segregated males—orange, ivory, orange reduced and ivory reduced as expected. Selection of two deep red of these "orange" males and crossing with ivory reduced females gave orange males in F_2 that were of the usual variability. No effect of selection immediately following mutation appeared.

Freaks 289, 319, and 308 may be regarded as mutants to orange among the expected four classes of males from $Oo^{i}Rr$ mothers.

Mosaic Male 286.—An F_1 female virgin from type female by ivory reduced male produced in addition to males—type 40, ivory 48, reduced 35, and ivory reduced 31, a black-eyed male with left wing reduced, right long (Freak 286), occurring in vial *d* (Fig. 1). He was set with seven different females over a period of eight days. He made prolonged and vigorous attempts to mate which appeared at times successful. No daughters were produced among 427 sons. Examination showed external genitalia to be abnormal

in the presence of an extra right external clasper, as also a curious chitinous structure suggesting an extra malformed penis (Fig. 2). Sterility of this male may be attributed to his inability to complete copulation. His potential breeding capacity is therefore unknown. It is postulated that the first oöcyte division was equational for Rr and that two oötids containing R and r respectively took part in cleavage.

Mosaic Male 287.—An F_1 female virgin from type female by ivory reduced male produced in addition to males—type 37, ivory 38, reduced 36, and ivory reduced 42, an ivory-eyed male (Freak 287) with right wings reduced, left long, occurring in vial d (Fig. 10). Eyes and ocelli were typical for ivory.

Freak 287 was mated to each of thirteen females over a period of fifty-two days. Daughters were produced by all of them. Three subsequent matings resulted in males only, 276, indicating sterility due to age. In addition to the 270 males of maternal type produced by the first thirteen females there were the following biparental offspring:

Six ivory reduced females produced 113 black reduced, 8 males and 105 females.

Three orange/ivory reduced females produced 120 black reduced, 5 males and 115 females, and 1 ivory long female.

Four homozygous orange reduced females produced 26 black reduced, 4 males and 22 females.

One homozygous ivory long female produced 24 type, 2 males and 22 females.

Freak 287 therefore had the capacity to produce biparental males, 19 to 265 females. Biparental males from recessive mothers indicate as do females what genetic factors are in the sperm. Black biparental offspring number 284 of which those with reduced mothers, 260, were all reduced. The single ivory long daughter from orange/ivory reduced mother represents the combination visible in the eyes and left wing of Freak 287.

Each of two of the reduced biparental males with ivory reduced mother was mated once. Ivory reduced males only resulted, indicating that biparental sons of mosaic males tend, like other biparental males, to be sterile.

The origin of Freak 287 may be expressed

First polar body	$Oo^{i}Rr$
Cleavage nuclei	*Or* $o^{i}R$

Italics are used to designate origin of gonads.

Mosaic Male 307.—Five type females, F_1 from ivory female (stock 17) by type male (stock 24) produced males only—type 412 and ivory 408 and in vial h a male (Freak 307) with pale orange eyes and ocelli typical for orange. He was mated with fourteen ivory females over a period of twenty-five days. Besides the 939 ivory sons there were 470 biparentals, all black, 9 males and 461 females.

Two of these biparental males were tested by mating, each to two females at an interval of eight days. Males only resulted, 66 and 104 respectively. Biparental males from Freak 307 are therefore of the usual sterile type.

Three daughters of Freak 307, produced males only, type 139, ivory 154.

Reduced is not concerned in the production of Freak 307. His origin may be expressed

First polar body	Oo^{i}
Cleavage nuclei	*O* o^{i}

If it be assumed that o^{i} mutated to o, Freak 307 is not only a mosaic but also a mutant. This question is discussed below.

Mosaic Male 321.—A black reduced male was mated to an ivory reduced female. 2 ivory reduced males and 28 black reduced females resulted. One of these daughters produced ivory reduced males 2, and a reduced male (321) with ocelli and left eye black, right eye black with red or orange area ventrally (Fig. 9). Long wings are not involved in this experiment. Freak 321 was mated to twenty-five ivory females over a period of twelve days. Ivory males 1816, black (biparental) males 66, and black females 806, resulted.

Freak 321 may be regarded either as a mosaic of black and ivory in which the ivory has taken on a reddish appearance due to proximity of "black" tissue or as a simultaneous mosaic and mutant. Its origin may be from a binucleate egg, or from a normal egg with subsequent mutation of O to o.

Mosaic Male 323.—A type male was mated with an ivory re-

duced female. Type females only (26) resulted. One of these produced 44 males—type, ivory, reduced, ivory reduced as expected and in vial *d* a male, Freak 323, with long wings and mosaic eyes and ocelli (Figs. 5 and 6). He was mated with sixteen ivory reduced females over a period of eight days. Ivory reduced males 158, type (biparental) males 16, and type females 280, resulted.

Freak 323 is comparable with Freak 321 in that light areas of eyes are orange rather than ivory. They are, however, much more extensive. His origin on basis of two oötids may be expressed:

First polar body	$Oo^{i}rr$
Cleavage nuclei	$OR \mid o^{i}R$

o^{i} may have mutated to o or the orange color may be purely somatic.

Mosaic Male 283.—An F_1 female from ivory reduced female by type male produced in addition to type males 50, ivory males 36, reduced males 38, and ivory reduced males 41, an orange-eyed male (Freak 283) with long wings occurring in vial *c*.

Two matings of Freak 283 with ivory long females (stock 17) resulted in 133 ivory males and 161 type females. The production of black-eyed daughters indicates that Freak 283 was a mosaic involving black. Fifteen of these type daughters isolated as virgin produced males—type 321, ivory 277, reduced 252, and ivory reduced 278. The production of reduced males proves that Freak 283 transmitted reduced and was therefore mosaic of long and reduced, as well as of black and ivory (or orange).

A mating of Freak 283 with orange reduced (stock 19) female resulted in orange reduced males 41, reduced females 56, showing directly that Freak 283 produced reduced only. Five of these reduced females produced reduced males 238, and orange reduced males 208, as expected. Freak 283 was also mated to type female (stock 1). This resulted in type males 93, and type females 30. Six of these females produced males—type 99 and reduced 83, and one which was mated to Freak 283 produced females—type 14, and reduced 15.

Freak 283 was also mated with three ivory reduced females. There resulted ivory reduced males 28, and reduced females 139, as well as a gynandromorph (Freak 304) with reduced wings and

eyes mosaic of black and orange discussed below. Eight of these females which had mated with their ivory reduced brothers produced males—reduced 30, ivory reduced 34, and females—reduced 93, and ivory reduced 92, as expected.

Summarizing the results of the eight matings of Freak 283 extending over a period of fourteen days, we find that he transmitted black, O, in at least 363 cases and reduced, r, in at least 232 cases. In other words he bred as a black reduced and no new factors or unusual types appeared except the gynandromorph, 304, discussed below. His origin on the basis of egg binuclearity may be expressed.

First polar body	$Oo^{i}Rr$
Cleavage nuclei	$Or \mid o^{i}R$

Freaks 283 and 307 differ from freaks 321 and 323, in that there is no trace of black in the eyes. Orange appearance is therefore not due to proximity of black facets, but to some physiological influence from "black" tissue or to mutation.

Mosaic Male 306.—An F_1 female from type female by ivory reduced male was mated with an orange long male (stock 12). From vials *a–d* there appeared males—type 9, ivory 13, reduced 6, ivory reduced 9, and females—type 26, and orange 27, as expected. Subsequently (vials *e–h*) there appeared males—type 26, ivory 26, reduced 34, ivory reduced 17, and a long-winged male with light orange eyes and mosaic ocelli appearing in vial *h* (Freak 306, Figs. 7 and 8). As may be seen from the figure the right ocellus is without pigment, even the slight amount characteristic of "orange" being absent. Anterior and possibly also left ocelli are mosaic. That the thorax is also possibly mosaic is indicated by its lighter color on right side.

Freak 306 was mated over a period of thirty-eight days with nine ivory reduced females and with five ivory long carrying reduced. In addition to the 814 males of maternal type there were 601 ivory long daughters. 464 of these (from ivory reduced mothers) prove that Freak 306 transmitted long. Of the remainder from Rr mothers, about half must have been long because of factor R in the sperm.

Freak 306 therefore breeds like an ivory long. The ocelli cer-

tainly contain black and either ivory or orange. The compound eyes are of definite orange appearance. Origin may be expressed

First polar body	$Oo^{i}rr$
Cleavage nuclei	$OR \mid o^{i}R$

"Black" tissue is regarded as present because of the ocelli. If orange appearance be due to mutation of o^{i} to o then there are three types of tissue here, O, o, and o^{i}.

Mosaic Male 305.—An F_1 female from type female by ivory reduced male was mated with an orange long male (stock 3). There were produced males—type 23, ivory 24, reduced 18, and ivory reduced 28, and females—type 94, and orange 74 as expected. There also appeared in vial *g* along with type and orange sisters, a male, Freak 305, with long wings, orange ocelli, and eyes mosaic of black and orange (Figs. 11 and 12).

Freak 305 was mated with eleven females over a period of twenty-nine days. Three subsequent matings resulted in males only, 297, indicating sterility due to age. The eleven females producing daughters gave in addition to 529 matroclinous sons, the following biparentals:

Three homozygous ivory females produced type females 6, ivory females, 122.

Eight ivory reduced females produced type females 19, ivory long male 1, ivory long females 243, ivory females with wings unexpanded 8.

Freak 305 therefore transmits long wings, 263 cases, both in association with black, 19 cases, and ivory, 244 cases.

Table 2 shows distribution of the 25 black and 374 ivory progeny according to age of Freak 305.

Origin of Freak 305 may be expressed

First polar body	$Oo^{i}rr$
Cleavage nuclei	$OR \mid o^{i}R$

Gonads are evidently mosaic for O and o^{i}, eyes for O and o^{i} or O and o.

Diploid Mosaics.

Mosaic Female 313.—An orange long female (stock 10) mated with an ivory reduced male (stock 20) produced orange long—

males 20 and females 6 and an orange female (Freak 313) with left wing reduced, right long (Figs. 22 and 23) and fifteen joints in each antenna, typical for female.

TABLE II.

DISTRIBUTION OF BLACK AND IVORY PROGENY ACCORDING TO AGE OF MOSAIC MALE 305.

Progeny.	Age in Days since Eclosion.											Total.
	4	5	7	12	13	16	18	26	28	30	32	
Black		4		1	5	2	2	1	10			25
Ivory	9	56	10	49	64	48	39	23	74	1	1	374

Freak 313 which had mated with her orange brothers produced males—orange 13, ivory 9, orange reduced 13, ivory reduced 10, and females—orange 39. Two of these females, when tested, produced reduced sons while five gave only long. Eggs of Freak 313 bearing R therefore numbered 27 while eggs bearing r numbered 25.

Freak 313 may be regarded as a heterozygous female, $oo^{1}Rr$, in which some somatic mitotic irregularity occurred eliminating R in the development of the left wing.

It may be noted that this mosaic female, unlike the mosaic males thus far discussed, had a homozygous mother.

Mosaic Biparental Male 318.—An ivory reduced female mated with an ivory long male produced ivory reduced males, ivory long (biparental) males, and ivory long females and ivory male (Freak 318) with left wing reduced, right wing long and terminal joints of right antenna fused (Figs. 3 and 4).

Freak 318 differs from mosaic males previously described in having a homozygous mother. It is evidently biparental, receiving R from its father. Its mosaicism was regarded as comparable in origin to that of female, 313 (somatic mitotic irregularity eliminating R). Being biparental, it was expected to be sterile or to produce a few sterile daughters. It was mated with six females over a period of twelve days. Nothing but males of maternal type appeared, totalling 946.

Gynandromorphs.

Gynandromorph 296.—An F_1 female from type female by ivory reduced male was mated with an orange reduced male (stock 19). There were produced males—type 23, ivory 29, reduced 28, ivory reduced 18, and females—type 34, orange 25, reduced 26, orange reduced 35, and in vial *c* a gynandromorph (Freak 296) with ivory eyes, right wing long, left reduced, sixteen joints in right antenna, 24 in left, and female abdomen (Figs. 18, 21). Measurements showed ocelli of male type. Responses resembled those of the male.

Since the father was orange the male parts (head with ivory ocelli and compound eyes) were of maternal origin. Difference in primaries indicates that one oötid contained R, the other r.

Gynandromorph 302.—An F_1 female from type female by ivory reduced male was mated to an orange reduced male (stock 19). There were produced males—type 14, ivory 17, reduced 10, ivory reduced 11, and females—type 39, orange 32, reduced 33, orange reduced 34, and in vial *f* a gynandromorph (Freak 302) with black eyes and ocelli, male head (right antenna with 20 joints, left with 21 and ocelli of male size), long wings and female abdomen. Responses were characteristic of the male.

Head and wings show maternal traits. Antennæ and ocelli and therefore presumably compound eyes are male. Whether wings are male like head (haploid) or female like abdomen (diploid) is uncertain.

Gynandromorph 303.—An F_1 female from type female by ivory reduced male was mated to an orange long male (stock 12). There were produced males—type 10, ivory 14, reduced 11, and ivory reduced 8 and females—type 36, and orange 46, and a gynandromorph (Freak 303) in vial *b* with ivory eyes and ocelli, long wings, male head, antennæ with 23 joints) and female abdomen (Fig. 24). Responses were characteristic of the male.

Eye color indicates maternal origin of male parts (head). Wing character may have been derived from either parent and may be either haploid (male) or diploid (female).

Gynandromorph 288.—An ivory reduced female mated to a light (black eyes, dilute ocelli), o^l, long male produced 4 ivory re-

duced males, 3 light long males, (biparentals), 23 light long females and a gynandromorph (Freak 288) in vial *c* with ivory eyes, male head (23 joints in each antenna), left wing long, right reduced, and abdomen female (Figs. 19 and 20). Pupal membrane adherred to tip of abdomen and specimen was nearly dead when found, perhaps diseased. Darker color of right side of sternum indicated that right side of thorax, with wings reduced (matroclinous) was probably male. This is consistent with fact that head, also matroclinous, is male.

Gynandromorph 322.—An ivory reduced female mated to a black reduced male produced 10 ivory reduced males, 46 black reduced females and in vial *b* an ivory reduced gynandromorph (Freak 322) with male head (20 joints in each antenna) and a female abdomen. Reactions were male but weak. Long wings are not concerned in the cross. Eye color shows male parts to be maternal in origin.

Gynandromorph 304.—An ivory reduced female mated to Freak 283, orange long male breeding as black reduced (see above) produced ivory reduced males 12, black reduced females 71, and in vial *b* a gynandromorph (Freak 304) with reduced wings and eyes mosaic for black and orange (Figs. 13–17). Left antenna is clearly female (15 joints), right is male but with terminal joints fused. Ocelli are mosaic, the right and anterior with considerable black pigment, predominantly female; the left with very little pigment clearly larger than the right and exclusively or predominantly male. Compound eyes are mosaic for black and orange. Abdomen is entirely female, sting split, and right gonapophysis, in agreement with right antenna, deficient. The left secondary is considerably longer than the right indicating femaleness.

Responses proved to be exclusively male. Repeated and vigorous attempts were made to mate with females.

Freak 304 is evidently an intricate mosaic of male and female parts, very different in this respect from any gynandromorphs previously found in this species.

The size of the left ocellus indicates that it is male and its reduced amount of pigment shows its maternal origin. Black regions of eyes indicate paternal influence and are assumed to be female (diploid). Orange regions of eyes are presumably male

(haploid) and of maternal origin, ivory being changed to orange at least in appearance.

Gynandromorph 325.—An ivory reduced female mated to a black reduced male produced 7 black reduced females and in vial *a* a small-sized gynandromorph (Freak 322) with male head and female abdomen. Eyes and ocelli were entirely black, the latter of male size. Wings were wrinkled with skin adhering. Abdomen was collapsed, genitalia immature. Sternites were of female type. Antennæ were deficient. The left had seventeen joints, the right eighteen with the two terminal fused. These antennæ are interpreted as male since counts of joints of over eighteen hundred female antennæ previously made showed none above sixteen and this number occurred in the larger individuals only.

The specimen was weak when found. Reactions could not be tested.

Freak 322 is the first gynandromorph found with male parts patroclinous. Male biparentalism is indicated by the general abnormality and weakness of the specimen. As explanation for origin, either egg binuclearity and dispermy or somatic mitotic irregularity may be suggested.

Frequency of Occurrence of Mutants and Mosaics.

Of the 14,023 wasps recorded in Table I., 9,787 (normal males 9,780, mutant males 3, and mosaic males 4) may be regarded as coming from unfertilized eggs and 4,236 (normal females 4,224, biparental males 9, and gynandromorphs 3) from fertilized eggs laid by diheterozygous mothers, $Oo^{i}Rr$. These mothers came in all cases from crosses of stock 20, ivory reduced, and a type stock, either 11 or 24.

Mosaic and mutant males and gynandromorphs number 9 among the 7,968 descended from stock 11, while there is but one mosaic male among the 6,115 descendents of stock 24. This may indicate a difference in hereditary tendency toward binuclearity in favor of stock 11.

Among the eleven haploid freak males above discussed, three (289, 308, 319) were mutants but not obviously mosaics, two (286, 287) were mosaics but not obviously mutants, and six (283, 305, 306, 307, 321, 323) were mosaics and at least apparently

mutants for they showed orange color in eyes. Since two (283, 307) at least of the latter would not have been tested and detected as mosaics had they not been orange, it was thought that other males apparently segregating in normal manner might be mosaic also. Gonads might differ from soma.

Accordingly F_2 males of normal appearance from a cross of ivory reduced (20) by type (11) were tested by mating to ivory reduced females. Thirty-five type bred as type (offspring totaling ivory reduced males 172, type males 24, type females 750), forty-two ivory bred as ivory (offspring totalling ivory reduced males 218, ivory males 28, ivory females 911), thirty-eight reduced bred as reduced (offspring totalling ivory reduced males 254, reduced males 21, reduced females 1,217) and twenty-nine ivory reduced bred as ivory reduced (offspring totalling 77 males and 285 females. Thus of 144 normally segregating males from $Oo^{i}Rr$ females none showed gonads different from soma.

Hypotheses Suggested.

The data above presented show that orange eye color may appear in males from eggs laid by mothers heterozygous for black and ivory, Oo^{i}, in at least two or possibly three different ways. Biparental males obviously receive the factor O from their father. Males with eyes mosaic for black and orange have thus far bred as black (321, 323), as ivory (306), or as black and ivory mosaic (305). In the case of three of these it may be supposed that proximity of black facets may cause the "ivory" to take on an orange appearance, but number 306 had compound eyes entirely orange with dark pigment appearing in the ocelli only. Moreover since two orange males (283, 470), breeding as black showed no trace of black pigment, it must be supposed that influence of "black" tissue in changing ivory to orange is not merely an optical effect from proximity of black facets, but is due to some physiological (enzymatic?) influence which actually transforms ivory to orange. If this be the case, it must be supposed that in number 287 (ivory breeding as black-ivory mosaic) the "black" tissue did not involve the "enzyme-producing gland."

An alternative to this "somatic" explanation is to suppose that actual genetic change has taken place. In the mosaic males gonads

have not included this orange tissue. It may be suggested that the three orange mutants (289, 308, 319), although not obviously mosaics, may have been derived from binucleate eggs carrying O and o^i for their mothers were Oo^i.

The hypothesis of mutation of o^i to o caused by association with O tissue brings the various phenomena into harmony. Evidence both that it is o^i rather than O which has changed and that this change has taken place in somatogenesis rather than in ovogenesis is afforded by the mosaic-eyed (black-orange) gynandromorph 304 in which O must have been derived from the male parent for the mother was o^io^i.

Evidence decisive between the somatic and the genetic hypotheses has not yet appeared. A mosaic male from Oo^i female producing o gametes or a male with eyes mosaic of orange and ivory would be of much interest in this connection.

Summary.

1. Independent segregation of ivory (eye color) and reduced (wings) is shown in progeny of Oo^iRr females.

2. When such females are mated to orange, o, males there occur among the normal offspring, orange (biparental) males with characteristic sterility.

3. One female from RR female by r male and one sterile biparental male from rr female by R male were each characterized by possessing one long and one reduced wing. Elimination of chromosome bearing R is suggested as explanation.

4. Eight mosaic males were produced by Oo^i females. Of these—one was sterile, four produced black daughters only, one ivory only, and two both black and ivory. It is suggested that these males arise from binucleate eggs and that gynandromorphs are produced when one nucleus of such eggs is fertilized.

5. Of seven gynandromorphs six showed male parts matroclinous while in one they appeared patroclinous. In the latter case they may have been diploid, comparable to ordinary male biparentalism.

6. Two mosaic males had eyes completely orange while four mosaic males and one gynandromorph had eyes mosaic of black and orange. Since presumably only black or ivory entered into

the gametes producing these mosaics, the orange color must have been due to some somatic physiological effect or to mutation.

7. Three mutant orange males showing no mosaicism and breeding as orange were produced by $Oo^{1}Rr$ females.

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'26 Quadruple Allelomorphs Affecting Eye-Color in Habrobracon. Am. Nat., LX, May–June. 286–290.

Whiting, P. W.
'26 Two Wing Mutations in *Habrobracon* and their Method of Inheritance. Am. Nat., LX., Sep.–Oct., 443, 454.

Whiting, P. W. and Whiting, Anna R.
'27 Gynandromorphs and other Irregular Types in Habrobracon. Biol. Bull., LII., 2, Feb.

EXPLANATION OF PLATE I.

FIGS. 1–24. Camera lucida drawings of various parts of mosaics of *Habrobracon juglandis*.

Figs. 1, 4, 10, 13, 18, 19, 22. × 10.

Figs. 3, 11, 12, 14, 15, 17, 20, 21, 23, 24. × 22.

Figs. 2, 6, 9. × 30.

Figs. 5, 7, 8, 16. × 63.

Figs. 1–12 are from mosaic males.

Figs. 13–21 and 24 are from gynandromorphs.

Figs. 22 and 23 are from a mosaic female.

Figs. 1 and 2, wings and ventral view of genitalia of mosaic male 286.

Figs. 3 and 4, right antennæ and wings of mosaic male 318.

Figs. 5 and 6, dorsal view of ocelli and anterior view of head of mosaic-eyed male 323.

Figs. 7 and 8, dorsal and anterior view of ocelli of mosaic male 306.

Fig. 9, right view of head of mosaic-eyed male 321.

Fig. 10, wings of mosaic male 287.

Figs. 11 and 12, dorsal and ventral views of head of mosaic-eyed male 305.

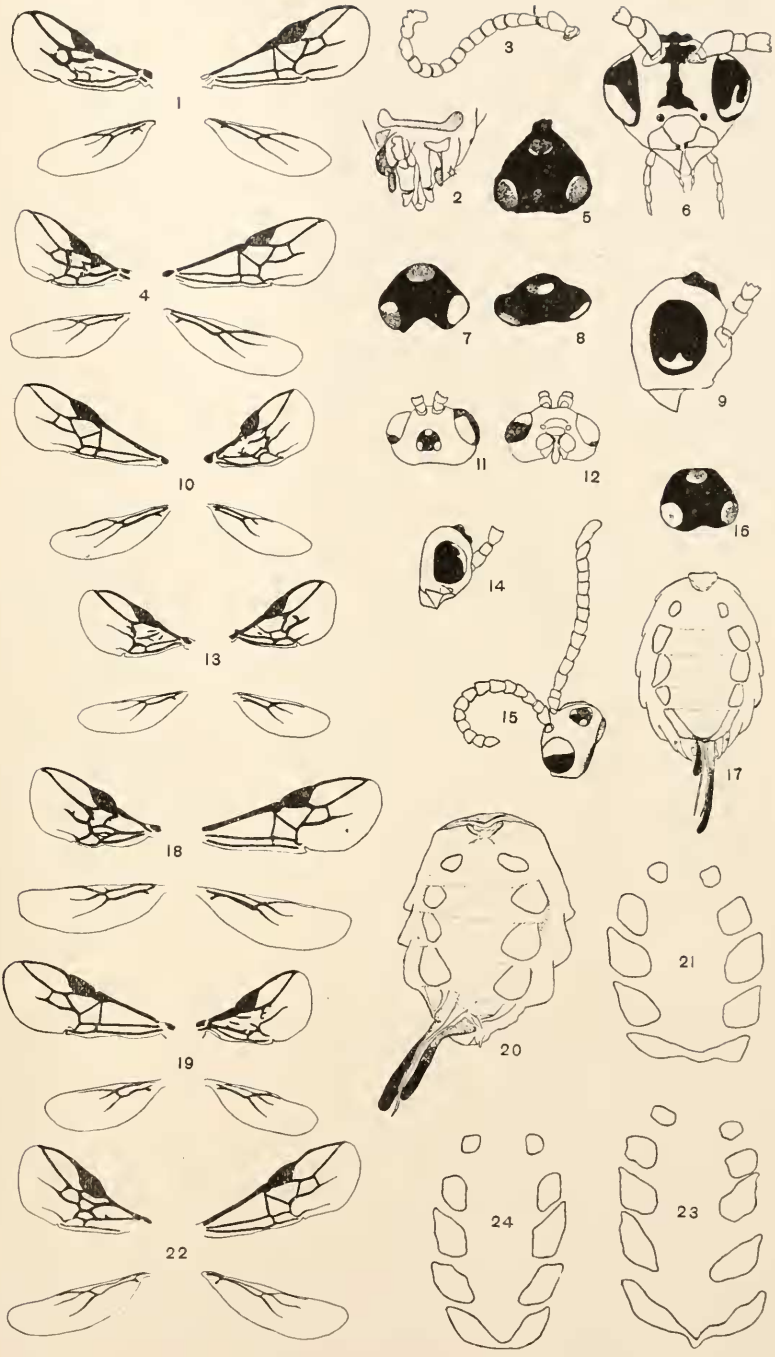
Fig. 13, wings, Figs. 14 and 15, right and dorso-sinistral views of head, Fig. 16, dorsal view of ocelli and Fig. 17, ventral view of abdomen of mosaic-eyed gynandromorph 304.

Figs. 18 and 21, wings and abdominal sternites of gynandromorph 296.

Figs. 19 and 20, wings and ventral view of abdomen of gynandromorph 288.

Figs. 22 and 23, wings and abdominal sternites of mosaic female 313.

Fig. 24, abdominal sternites of gynandromorph 303.



P. W. WHITING.

OVULATION IN THE FOUR-TOED SALAMANDER, *HEMIDACTYLIUM SCUTATUM*, AND THE EXTERNAL FEATURES OF CLEAVAGE AND GASTRULATION.

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Introduction.

Incidental to the collection of material for a study on the germ cells of *Hemidactylium scutatum* the writer brought to the laboratory a female of this species which subsequently deposited a number of ova while under observation. The eggs laid proving fertile, the progress of cleavage and gastrulation was carefully noted. Though the observations made cover but a very limited material, they serve to supplement the work of Bishop ('18) who begins his account of the development of Hemidactylium with the neural plate stage; moreover, ovulation in this species seems not to have been previously observed and reported. For these reasons the observations made are here reported, even though the limited material prevents consideration of but little more than the superficial features of cleavage and gastrulation.

Ovulation in *Hemidactylium*.

The female on which these observations were made was captured in the sphagnum bog on the shores of Mud Pond near Ithaca,[1] the site of Bishop's discovery of the egg-laying habits of this species some years before. This particlar female was found in a sphagnum hillock together with two eggs, on the morning of May 11, 1923. She was brought to the laboratory the afternoon of that day, and placed in a small glass dish of sphagnum, to-

[1] This study was carried on in the laboratories of the Department of Histology and Embryology of Cornell University. The writer wishes to acknowledge the kindness of Dr. B. F. Kingsbury at whose suggestion the observations herein reported were undertaken.

gether with the eggs assumed to be hers. About 9 P.M. of the same day it was noted that eight eggs were present, all but three of which were scattered over the bottom of the dish. The female had been partially concealed by the sphagnum, and was of course necessarily disturbed by the attempt to locate her and her eggs for observation. It would seem, however, that her position when first accurately observed was probably but little changed, since she was in contact with two ova which later observations would indicate had been but recently extruded. She lay with her back turned against the bottom of the dish, her abdomen upturned against the overlying sphagnum. Two eggs lay above her, still in contact with her ventral body wall immediately cephalad of the cloacal opening, but adherent to the overlying sphagnum; by gently pulling upon this sphagnum the eggs could readily be pulled away from the body of the female, who was not at all disturbed by this procedure, but remained quietly in position. Her body was somewhat curved, so that the middle part of her abdomen was in contact with the side of the dish; the end of her tail also lay in contact with the side of the dish, elevated along it and not in contact with the bottom.

With the body of the female in this position, it was noted at 9:14 P.M. that an egg was beginning to appear at the cloacal opening. The female made no unusual movements, but lay quietly while the egg continued to move slowly outward. In about two or three minutes it was practically expelled from the cloaca, but the female lay motionless for several minutes before a slight shift of her body left the egg attached to the overlying sphagnum.

The process of ovulation was not repeated until about 10:55 P.M. During the expulsion of this second egg the female twisted the front half of her body in such fashion that her left side was toward the bottom of the dish; the caudal part of the body however, was kept in its former position, with the dorsum against the bottom of the dish and the vent towards the overlying sphagnum. This position was retained for several minutes following the expulsion of the egg. After about twelve minutes the female was brought into a brighter light for more careful observations, and thereupon moved slowly away from the position she had maintained throughout the expulsion of the two ova. She now lay

with the abdomen pressed against the side of the dish, the cephalic third of her body in a vertical position, the remainder, except for the end of the tail, extending in a horizontal direction along the junction of the side and bottom of the dish. In this position a third egg was expelled. As in previous ovulations, no unusual movements of the female were noted during the process. Preceding the appearance of the egg at the cloacal opening the female was seen to straighten her body and move the hind limbs slightly—movements perhaps necessary to facilitate the passage of the egg through the pelvis. The female then lay motionless with the abdomen pressed against the side of the dish while the egg was slowly forced from the cloacal orifice and against the glass. After the female had remained for about eight minutes with the egg in this position she was gently pushed away from the side of the dish, to which the egg remained adherent. As she showed no indication of further attempts at ovulation, the observations were discontinued. This female subsequently deposited a few other ova, but at times when she was not under observation.

The position of the female during the expulsion of the first two ova is of considerable interest, since it may possibly represent an approach to the normal. A horizontal position of the body with the ventral surface uppermost at the beginning of ovulation would prevent the eggs from dropping down into the spaces of the sphagnum, and would insure the contact and adherence of eggs successively expelled from the cloaca, since each egg as it leaves the cloacal opening would be supported upon the body of the female in contact with the egg previously extruded. With the accumulation of a number of eggs, the size of the egg mass, together with the viscid character of its surface would effectively prevent its dropping downward in the spaces of the sphagnum. Females taken in sphagnum bogs after ovulation ordinarily have their eggs loosely arranged in a mass around or over which the body of the animal is coiled.

The eggs when first expelled from the cloaca show only an extremely thin gelatinuous capsule; after several minutes this is noticeably thicker, due to the absorption of water from the moist environment. The outermost layer of this jelly is of a semi-fluid, viscid nature, which causes the eggs to adhere readily to each

other or to sphagnum, glass, or other gelatinous egg envelopes, while a thin, membrane (vitelline membrane) closely surrounds the egg or embryo and separates it from the more fluid gelatinous envelope; a similar membrane of the egg of *Cryptobranchus* is stated by Smith ('26) to be identical with the zona pellucida of ovarian eggs.

Fertilization in this species is internal, as in other Urodeles. The breeding habits of the animals are quite unknown to the writer, but since males have not been found with the females near ponds at the time of ovulation (Bishop, '18; Blanchard, '23) it may be concluded that mating occurs at some previous time, and very probably in a terrestrial environment, the transfer of sperm from male to female possibly being effected by contact of the ventral surfaces of the animals. Bishop quotes C. and H. Thompson ('12) and Moesel ('18) to the effect that males and females have been taken from beneath the same log or stone during the spring months.

The female possesses the sperm-storing organ or spermatheca characteristic of Urodeles. According to Dieckmann ('27) the spermatheca of this species is unique in that it consists of but a small number of spermathecal tubules opening directly into the cloacal chamber; the latter feature is regarded as a primitive one, while the former (reduction of tubules) is characteristic of the most highly developed spermathecæ, in which the tubules open to a single duct-like tube (common tube,—a modified portion of the cloacal chamber). Dieckmann's descriptions of the cloaca and spermatheca of this species are based upon two specimens captured in August near Buffalo, New York. In one of these, spermatozoa are very abundant, in the other absent; on the assumption that *Hemidactylium* males are incapable of mating during the summer months, as are practically all Urodeles, these spermatozoa must be interpreted as having been stored for at least several weeks after a mating in the spring months. Their large number and whorl-like arrangement in the spermatheca suggest however, that no ovulation had occurred after they had been received into the spermathecal tubules.

Cleavage.

The eggs laid under observation in the laboratory, together with others deposited by the same female, proved to be fertile, making possible a study of the early cleavage divisions. While the limited number of eggs necessarily prevented fixation and sectioning of successive cleavage stages, the external features of segmentation were carefully noted over a period of several hours, or until gastrulation was in progress.

In order to follow the progress of cleavage in individual eggs the ova were removed singly to numbered Syracuse watch glasses each containing a few drops of water; each egg was left in contact with a small bit of sphagnum rather than in the water. The watch glasses were transferred to the stage of a binocular microscope for observations; the position of the egg was changed as desired, either by moving the sphagnum to which it was attached, or by direct manipulation with needles. Though it was realized desirable to handle the eggs as little as possible, the difficulty of keeping them in position for viewing the vegetal hemisphere necessitated considerable manipulation. Though possibly this and other environmental factors may have contributed to certain of the atypical features of cleavage observed in some of the ova, it is nevertheless certain that eggs showing such features in some cases gave rise to normal embryos. The eggs were of course kept under observation without removal of their protective membranes.

Some of the eggs, when first viewed under the microscope, still showed the polar bodies adherent to their surfaces; these as a rule became indistinguishable after the first two or three cleavage divisions had been completed. The stage of maturation at the time of laying was not determined.

Since in none of the eggs laid in the laboratory during the night of May 11–12 did the first cleavage furrow make its appearance later than 10:30 A.M. of the following day, cleavage may be assumed to begin within from ten to fifteen hours after the egg is deposited. The egg of *Hemidactylium* apparently agrees with that of *Spelerpes* (*Eurycea*) in this respect (see Goodale, '11).

The pattern of cleavage will first be described in an egg in which the divisions proceeded in a relatively orderly, symmetrical fashion. In this egg the first cleavage furrow was first noted at

9:30 A.M.; it then extended over about a sixth of the egg circumference (see Fig. 1, *a*). From its middle point at the animal pole a short broad furrow extended at right angles to it on either side, the two furrows together forming a rather distinct cross. The short transverse furrow subsequently disappeared, being practically invisible by the time the first cleavage furrow had reached the egg equator. A similar furrow was observed in only one other egg. In another several short furrows radiating from the animal pole were formed preceding the appearance of the first cleavage furrow and disappeared after the latter had extended over a fourth of the egg circumference.

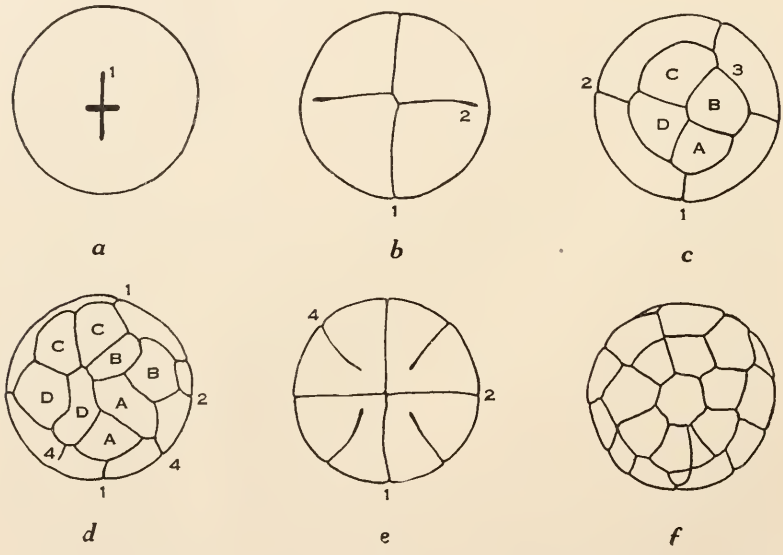

FIG. 1. Egg showing the most regular or symmetrical cleavage pattern observed. Viewed from animal pole except in *c;* cleavage furrows as numbered. *a,* 9:30 A.M. 5–12–23; *b,* 12 M.; *c,* 2 P.M.; *d,* 3:40 P.M.; *e,* vegetal hemisphere at 5 P.M.; *f,* 8:30 P.M. The origin of the micromeres in *d* is indicated by the lettering.

The progress of the first cleavage furrow through the more heavily-yolked vegetal hemisphere of the egg was at a much slower rate than its formation between animal pole and equator. From this egg and others observed we may conclude that the furrow is usually completed (on the egg surface) within about two hours after its first appearance.

The second cleavage furrows made their appearance before the first had been completed. In Fig. 1, *b* they are shown extending practically to the equator of the egg, 2½ hours later than the stage of Fig. 1, *a*. The first cleavage furrow in this egg is now complete. The second cleavage furrow apparently reaches the vegetal pole of the egg in from two to three hours.

The third set of cleavage furrows appeared about an hour after the stage of Fig. 1, *b*, and were completed within an hour, the egg at 2 P.M. having the appearance shown in Fig. 1, *c*. These furrows were roughly parallel with the egg equator and lay well up in the animal hemisphere. The four micromeres thus formed rotated a short distance in a clockwise direction to the position they occupy in Fig. 1, *c*.

The cleavage furrows of the fourth set were completed in the micromeres at 3:40 P.M., though just beginning to appear at that time in the macromeres. The direction of these furrows in *B* and *D* was roughly parallel with the first cleavage furrow; in *A* it was at right angles to this furrow, and in *C* it was meridional. With the completion of the division the daughter micromeres underwent readjustments of form and position such that the egg at 3:40 P.M. presented the appearance of Fig. 1, *d*. The fourth cleavage furrows of the macromeres are shown in this figure at an early stage of their formation; they had not been completed when the egg was sketched at 5 P.M. (Fig. 1, *e*). Their direction, while approximately meridional, was such that they would end in contact with the second cleavage furrow a short distance from the vegetal pole.

No further observations on this egg were made until 8:30 P.M., at which time it presented the appearance indicated in Fig. 1, *f*. It was impossible at this time to determine accurately the relation of any of the micromeres to those of Fig. 1, *d*.

It would appear from the above that cleavage in *Hemidactylium* is similar to that in the egg of *Eurycea* (Goodale) and is of the holoblastic unequal type characteristic of the moderately-yolked eggs of Urodeles. As in *Eurycea* and other species, however, the cleavage divisions conform to a symmetrical pattern for but few divisions if at all, and the blastomeres soon become of such irregular form and arrangement that their descent from any particular cell of the four- or eight-celled stage can not be determined.

Though irregularity of the cleavage pattern may be expected to appear during the third or fourth division in any egg of this species, as shown in Fig. 1, *d*, it is of earlier origin in a great many cases. Of ten eggs in which the furrow pattern could be determined for a stage equivalent to that of Fig. 1, *b*, in only four did it conform to this plan, and of these only one egg showed in the third division the regularity of pattern illustrated in Fig. 1, *c*.

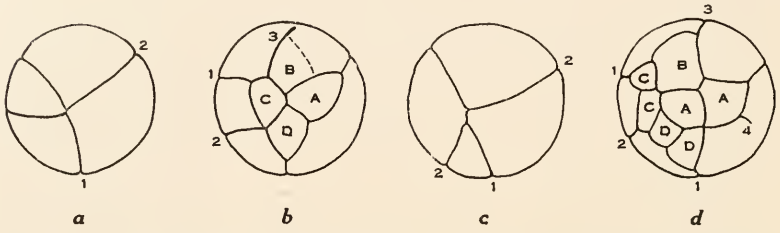

FIG. 2. Egg showing cleavage pattern resulting from inequality in the first division. Viewed from animal pole except in *c;* furrows numbered and origin of cells in *d* shown by lettering. *a*, 1 : 15 P.M., 5–12–23; *b* and *c*, 3 : 25 P.M.; *d*, 5 P.M.

Irregularity or asymmetry of cleavage in two of the ten eggs began with the first division. In one case it consisted merely in an inequality in the division of the egg (see Fig. 2, *a*); in the second division the larger of the two cells was divided more slowly than the smaller, the cleavage furrow in it reaching only thirty degrees below the egg equator when division in the smaller cell was completed. The third cleavage furrows were horizontal in three cells; in the fourth the furrow took a meridional direction, as shown in Fig. 2, *b*, in which it reaches almost to the egg equator. The vegetal hemisphere of the egg now appeared as in Fig. 2, *c*. A cleavage furrow along the dotted line of Fig. 2, *b*, subsequently cut off a micromere from cell B; this, with the division of micromeres *A*, *C*, and *D*, formed a group of seven cells of very unequal sizes arranged as in Fig. 2, *d*. The descendents of micromeres *C* and *D* formed a group of cells still distinguishable by their smaller size several hours later, in the fine-celled blastula stage; they then lay just above the egg equator. This egg subsequently completed gastrulation in the normal manner.

In the second egg to show irregularity beginning with the first division, the first furrow to appear had the position illustrated in Fig. 3, *a*. Within two hours other furrows of irregular direction had developed, giving the animal hemisphere the appearance of Fig. 3, *b*. At this time no furrows extended below the equator. Later several furrows extended into the vegetal hemisphere (Fig.

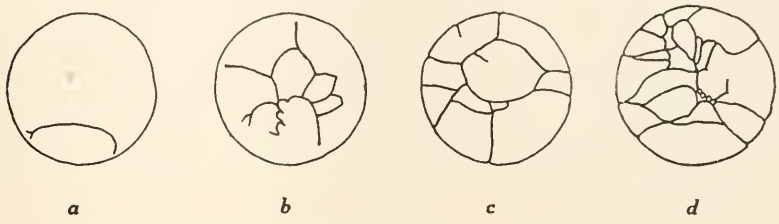

FIG. 3. Egg showing extremely irregular cleavage pattern beginning with the first division. Viewed from animal pole except in *c*. *a*, 2:45 P.M., 5–12–23; *b*, 4:45 P.M.; *c*, 8 P.M.; *d*, 10:25 P.M.

3, *c*). Fig. 3, *d* illustrates the appearance of the animal hemisphere in this egg about eight hours after the beginning of cleavage. Though continuing development for 24 hours longer and reaching a fine-celled blastula stage this egg finally died without beginning gastrulation. Its markedly atypical cleavage, therefore, may

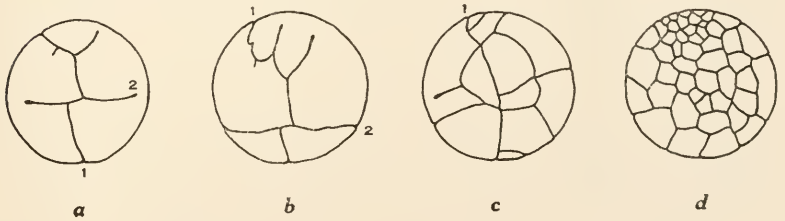

FIG. 4. Egg showing irregular cleavage pattern. Viewed from animal pole; egg in *b* is tilted to show full extent of atypical furrows. *a*, 10:25 A.M., 5–12–23; *b*, 12 M.; *c*, 1:45 P.M.; *d*, 10:45 P.M.

well have been the result of injury from drying, handling, or other causes.

Another egg with almost as pronounced irregularity of cleavage as that illustrated in Fig. 3 nevertheless gave rise to a normal embryo. In this egg the first cleavage furrow developed normally, and had completely encircled the egg within three hours.

Before the usual second furrows made their appearance, a furrow was noted leading from the first a short distance above the egg equator. About a half-hour later the appearance was as in Fig. 4, *a*, with the second furrows now present. Later the egg showed other furrows in the region in which the first atypical one had made its appearance (Fig. 4, *b*) and about five hours after the onset of cleavage the animal hemisphere displayed the atypical pattern of Fig. 4, *c*. In the somewhat later blastula stage illustrated in Fig. 4, *d* this egg showed a group of distinctly smaller micromeres at and below the equator in the region of the first atypical furrow. Though this peculiarity persisted until late in cleavage this egg completed gastrulation in the normal fashion.

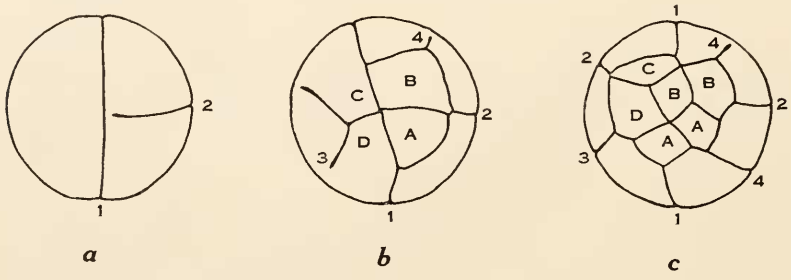

FIG. 5. Egg showing irregular cleavage pattern starting with retardation of one of second furrows. Viewed from animal pole except in *a;* furrows as numbered. *a* and *b*, 10:30 A.M., 5–12–23; *c*, 12 M. Origin of cells in *c* indicated by lettering.

Probably the most common type of irregularity initiated during the second cleavage division is that resulting from retarded or atypical formation of one of the two furrows of the set, so that one reaches the vegetal pole long in advance of the other, as illustrated in Fig. 5, *a*. The animal hemisphere of this egg pictured in Fig. 5, *b* shows that the third cleavage furrows were likewise retarded in the same half of the egg, they being completed in the half on the right while only one had appeared in the opposite hemisphere, assuming that the furrow marked 3 represents a furrow of the third set. In a stage corresponding to that of Fig. 1, *d* the polar cap of micromeres consists of but six cells (Fig. 5, *c*) instead of the eight found in the regular type of cleavage. This egg subsequently developed into a typical fine-celled blastula in which stage it was fixed for sectioning.

In many eggs the cleavage pattern becomes irregular in the course of the third division, after the first two divisions have proceeded in the regular or symmetrical fashion illustrated in Fig. 1. This irregularity may originate through retardation of division in one or more of the blastomeres, as illustrated in Fig. 6, *a*, in which only two micromeres are shown completely separated from the corresponding macromeres. Often, however, the irregu-

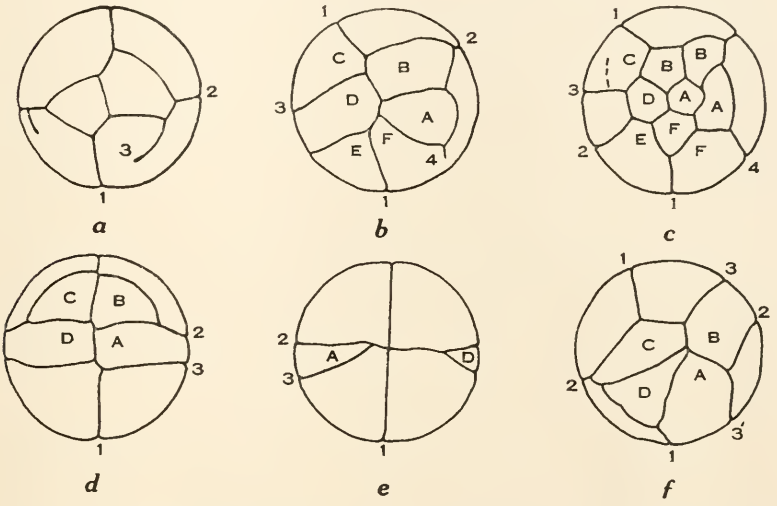

FIG. 6. Eggs showing irregularity of cleavage pattern beginning with third division, after first two have proceeded as in Fig. 1, *b*. Viewed from animal pole except in *e;* cleavage furrows as numbered. *a*, cell with all furrows of the third set horizontal, but furrows delayed in two of the blastomeres. *b* and *c*, egg with two furrows of third set atypical in direction; *b* at 11:45 A.M., *c* at 12:50 P.M. *d* and *e*, egg with two furrows of third set horizontal and the other two parallel to the second furrows in the animal hemisphere (*d*) but meeting the second furrows below the equator (*e*). *f*, egg with furrows marked 3 and 3′ atypical in direction.

larity results from the atypical direction of one or more of the cleavage furrows of the third set. In the egg shown in Fig. 6, *b*, for example, the line marked 3 represents a furrow of the third set which takes a direction parallel with the second furrow, and hence does not cut off a polar micromere in the usual fashion; the third furrow around micromere *A* is also atypical in direction, since it curves back almost to the animal pole instead of joining the first cleavage furrow nearer the equator. In the fourth cleavage

division of this egg micromeres *A* and *B* were divided transversely (see Fig. 6, *c*) and micromeres were cut off from cells *D* and *F*, giving a polar cap of six micromeres, to which a seventh was added later by division of blastomere *C*. The plane of this division is indicated by a dotted line in Fig. 6, *c*. A somewhat more symmetrical pattern was exhibited by the egg shown in Fig. 6, *d*; in this egg two furrows of the third set take the usual position parallel to the equator, while the other two run parallel to the second furrows in the animal hemisphere of the egg but join these furrows in the vegetal hemisphere (Fig. 6, *e*); cells *A* and *D* are thus not typical polar micromeres but extend well toward the vegetal pole of the egg. Still another egg with atypical cleavage furrows of the third set is shown in Fig. 6, *f*. In this egg two furrows of somewhat typical direction had cut off the micromeres *C* and *D*, while the furrow marked 3, meeting the second furrow below the equator, had cut off a much larger cell (*B*). The furrow 3′ ultimately joined the first cleavage furrow some distance below the equator, to cut off another large cell (*A*). This egg thus resembles that of Fig. 6, *d* in possessing only two micromeres of the usual size in the eight-cell stage.

Although only a limited number of eggs were followed through early cleavage, it would seem that irregularity or asymmetry of cleavage pattern in *Hemidactylium* probably begins with the third division in the majority of cases, but with even the first or second in some cases.

Though cleavage in *Hemidactylium* follows the general plan described by Goodale ('11) for *Spelerpes* (*Eurycea*) the vegetal hemisphere of the egg is earlier divided into small cells than in the latter species. According to Goodale the cleavage furrows of the fourth set in *Spelerpes* "do not even reach the lower pole, but usually join the earlier furrows less than 45 degrees below the equator." As a result of this mode of division, the egg of *Spelerpes* in a later stage may show 130 cells visible from above and only 7 from beneath, or 400 above and 14 beneath. In *Hemidactylium* the egg is less heavily yolked than in *Spelerpes* and less markedly telolecithal. The cleavage furrows of the fourth set, though not necessarily reaching the vegetal pole, nevertheless end farther below the equator (see Fig. 1, *e*), while eggs showing 7

or 8 cells when viewed from the vegetal pole show only 25 to 30 when viewed from above. These facts indicate that the egg of *Hemidactylium* is intermediate between that of *Amblystoma* and *Spelerpes,* and far less markedly unequal in its cleavage than the relatively large eggs of *Desmognathus* (Hilton, '09) or *Cryptobranchus* (Smith, '12). In the latter forms the tendency is for the third cleavage planes to be radial or meridional in direction, and the relatively small micromeres of the animal pole arise by later divisions; in *Hemidactylium,* on the other hand, the third cleavage furrows are more frequently equatorial (latitudinal) in direction, and cut off micromeres of relatively larger size, the egg in this respect resembling that of Amblystoma.

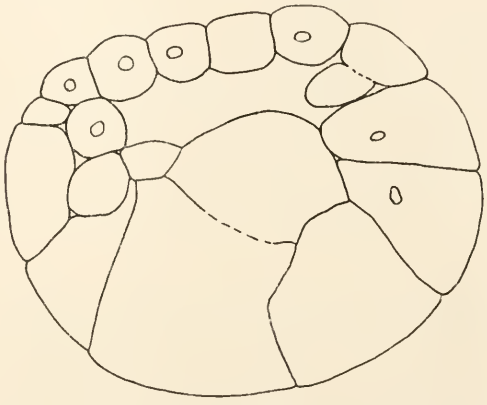

Fig. 7. Early blastula of *Hemidactylium* in vertical section. Outlined with camera lucida. × 31.

So few eggs were available that it was impossible to make a careful study of the internal changes of cleavage. Blastulæ of but two stages were sectioned. The earlier of these, represented in Fig. 7, shows a distinct segmentation cavity roofed by a single layer of cells. It is probable that the blastocoele makes its appearance early, as in the eggs of *Cryptobranchus* (Smith, '26) or *Desmognathus* (Hilton, '09), and as a distinct cavity rather than a collection of intercellular spaces such as Goodale states is frequently the condition in *Spelerpes.* Older blastulæ show a rather characteristic loose arrangement of the internal blastomeres, but a distinct blastocoele is nevertheless present.

Gastrulation and Closure of the Blastopore.

The earliest indication of gastrulation appeared about 90 hours after ovulation in the form of a crescentic depression well down in the vegetal hemisphere (Fig. 8, *a*). This crescent blastopore is bordered by a dorsal lip frequently showing a greater pigmentation than the neighboring surface. The crescent blastopore is gradually extended laterally through further invagination of cells

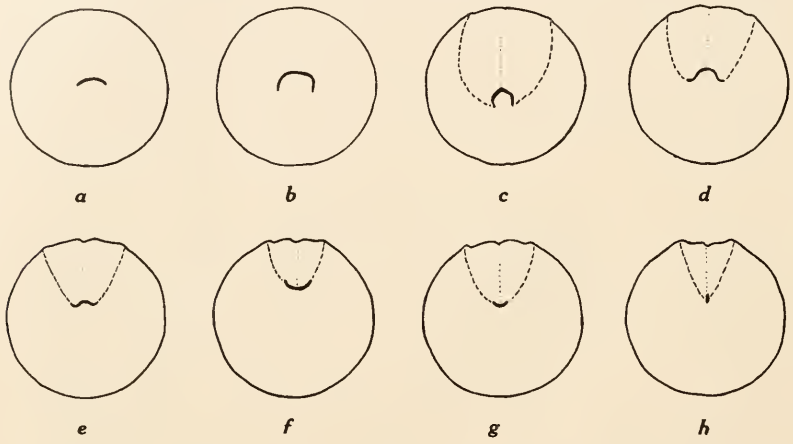

Fig. 8. Diagrams illustrating the formation and "closure" of the blastopore in *Hemidactylium*. No attempt is made to indicate exact relation of blastopore to egg equator. Margin of neural plate indicated by broken lines. *a*, early crescent blastopore; *b*, later crescent blastopore (from another egg); *c*, blastopore of *b* about 24 hours later; *d*, blastopore about 11 hours later than in *c*; *e*, later blastopore from another egg; *f*, blastopore of *e* about 9 hours later; *g*, smaller blastopore from another egg; *h*, blastopore of *g* 10 hours later, reduced to short vertical slit which persists as anus.

at its lateral margins (Fig. 8, *b*) until it assumes the form of a half circumference; sometimes it may become more extensive, as in Fig. 8, *c*, but in no case was the line of invagination or overgrowth observed to become a complete circle surrounding a yolk plug. Following its period of greatest ventral extent the blastopore shortens and assumes the form of a crescent with laterally directed margins (Fig. 8, *d*); by further narrowing and elevation of its lateral portions it assumes the form shown in Fig. 8, *e*, and finally the form of an inverted crescent (Fig. 8, *f*). As the neural folds close the blastopore gradually shortens (Fig. 8, *g*)

and finally assumes the form of a slit elongated in the direction of the body axis (Fig. 8, *h*). It does not appear that the blastoporic opening is lost but rather that it persists as the anal opening.

From the above brief account of the changes in form of the blastopore it may be seen that gastrulation in *Hemidactylium* closely resembles that in *Spelerpes* (Goodale, '11) so far as external features are concerned. Presumably the egg would show the same movements of surface material that Goodale was able to demonstrate in *Spelerpes* by the use of Nile Blue Sulphate; the egg is so lightly pigmented that it, like the egg of *Spelerpes,* might readily be used for studies of this sort. The conclusions of Goodale as to the formation of the germ layers in *Spelerpes* are probably likewise applicable to *Hemidactylium.*

Hemidactylium and *Spelerpes* are possibly unique among American Urodeles in lacking the "yolk-plug stage" characteristic of the development of Amblystoma and the *Anura.* In the larger, yolk-rich eggs of *Necturus* and *Cryptobranchus* the blastopore assumes the form of a complete circle (Smith, '12). Although Hilton claims this to be true also for *Desmognathus,* his Fig. 30 shows a late blastopore of the inverted crescentic form characteristic of the same stage in *Spelerpes* (Goodale, Pl. 1) or *Hemidactylium* (Fig. 8, *f* and *g*). Unfortunately observations on this stage of development of the eggs of *Gyrinophilus* and the Plethodons are not available; possibly in these species gastrulation would follow the pattern found in *Hemidactylium* and *Spelerpes.*

In his account of the development of *Hemidactylium,* Bishop ('18) estimates that his youngest specimen, an embryo showing well-elevated neural folds, is not more than 72 hours old. This estimate would appear to be much too low, assuming that the eggs timed by the writer had not been retarded by the conditions to which they were subjected. In these eggs the early crescent blastopore was first observed about 90 hours after ovulation, and the neural folds did not close until from 60 to 80 hours following this stage, or at from 150 to 175 hours after ovulation. Since temperature determines to a great extent the rate of the developmental processes, and since the laboratory temperature averaged higher than that of the normal environment, it is probable that the eggs observed developed at least as rapidly as would eggs in the field.

For stages of development later than those of Fig. 8 the reader is referred to the excellent sketches published by Bishop ('18). Transections of older embryos of this species are figured in a previous report dealing with the primordial germ cells (Humphrey, '25).

Summary and Conclusions.

1. The egg complement of the *Hemidactylium* female probably requires a period of several hours for its deposition, since the expulsion of a single egg occupies several minutes.

2. The observed position of the body of the female during ovulation (ventral surface upmost) may be of value in preventing scattering of the eggs, since they are thus supported upon the body of the female as well as by adherence to neighboring sphagnum or other eggs.

3. Under laboratory conditions cleavage begins in from ten to fifteen hours after ovulation; it is of the unequal holoblastic type. The first and second cleavage furrows tend to be meridional in direction. The furrows of the third set are frequently horizontal (latitudinal) and lie well above the egg equator, but in many cases they take a direction parallel with the first or second furrows in the animal hemisphere and meet these furrows at some point below the equator. A well developed blastocoele is very early present.

4. Irregularity in cleavage is to be expected in all eggs after the third division. Only occasionally do the eggs show symmerical eight-cell stages, due to the fact that irregularity or asymmetry in cleavage may begin with any of the first three divisions.

5. The vegetal hemisphere of the egg undergoes more division than in the eggs of *Spelerpes, Desmognathus,* or *Cryptobranchus.* In this respect it resembles the egg of *Amblystoma.*

6. Gastrulation begins about 90 hours after ovulation with the formation of the usual crescent blastopore. No ventral lip develops and no yolk plug is formed. The crescent blastopore shortens and becomes inverted, the horns of the crescent pointing upward, and finally closes to a vertical slit which becomes the anus.

7. Closure of the neural folds occurs in from 150 to 175 hours after ovulation. The further course of development has been described by Bishop ('18).

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FERTILE TERMITE SOLDIERS.

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In two earlier studies (Heath, '03, '27) attention was called to the fact that in the termite genus *Termopsis,* represented in California by two species (*T. nevadensis* and *T. angusticollis*), occasional individuals occur which externally resemble a soldier and yet are sexually mature. In the typical soldier the head is distinctly longer than broad, whereas in the fertile type the two diameters are the same or nearly so. Furthermore the jaws of both project far beyond the labrum, and are built upon essentially the same plan. Hence, unless it is decided that they belong to a special caste, these unusual insects must be looked upon merely as soldiers with fully developed sex organs.

Up to the time when the manuscript of the later paper went to press only four of this type of termite had been discovered. Since then fourteen additional individuals have been collected, and it now appears that they may prove to be of rather frequent occurence. All of these later acquisitions were living in essentially the same conditions. A limb of a Monterey pine (*Pinus radiata*), ten or fifteen feet in length, and evidently occupied by a colony of *T. nevadensis* throughout its entire extent, had broken off near its base, and in falling had broken into several fragments. When the weathering of these, and the depth they had sunk into the leaf mold indicated a sojourn of several months each fragment was carefully opened. A large number of such colonies were examined, and while in most instances complemental royal forms were present, those with soldier-like appearance existed in approximately twelve per cent. of the communities.

In addition to their relatively small heads the behavior of these fertile soldiers is unmistakable. Unlike the typical soldier, which moves about from place to place in the observation nest, the fertile type remains in close association with the normal complemental in-

sects. Also the females are readily distinguished by their distended abdomens. Conjugation was witnessed on six occasions where the soldier paired with an individual of the usual neoteinic type. No especial attempt was made to observe the actual egg-laying

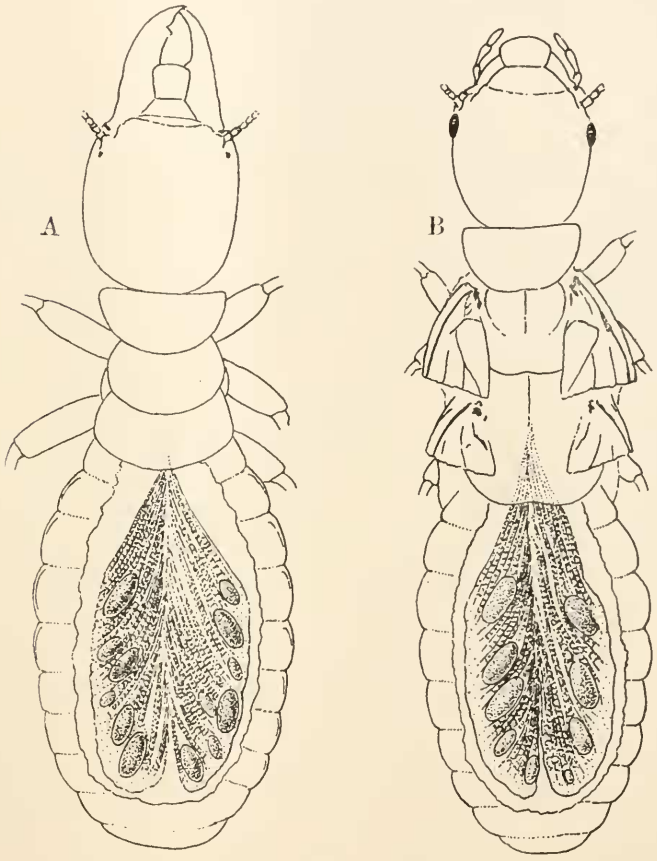

FIG. 1.

process, since this had been observed on two former occasions. Dissections, however, of six specimens showed the sex organs to be well developed. In the text figure, for example, a specimen of female complemental soldier is compared with a primary queen from a colony of *T. nevadensis* two and one half years old. In the first named individual (*A*) there were seven fully-formed ova, including one in the oviduct (not represented), while in the other

specimen the ovary contained eight fully-developed eggs. In addition there were approximately the same number of developing ova in both insects.

In a former account (Heath, '27) some observations were recorded which indicate that the complemental royal forms, at least in the genus *Termopsis*, are fertile soldier nymphs. The fertile soldier also points to the same conclusion. Under normal circumstances it appears that in an orphaned colony insects of this type appear as a rule to be in the penultimate instar, and yet there are occasional specimens which give evidence of belonging to the antepenultimate stage. Also it appears to be true that in some instances the sex gland stimulus may be applied to individuals which have progressed so far along the path to complete soldier development that with the functional activity of the gonad there is a slight suppression only of those characters which normally belong to the fully-developed soldier. This seems to be a reasonable explanation of the origin of these individuals, which are thus seen to combine the activities of the usual complemental royal insect together with many of the structural features of the typical soldier.

Up to the present time no essential differences have been discovered between the offspring of the primary royal pair and those of the complemental royal forms including the fertile soldier. The data relating to this last named category, however, are based upon the study of two colonies only. A report upon a more extensive series of experiments, now being conducted, will be made at a later date.

REACTIONS OF THE CYPRID LARVÆ OF BARNACLES AT THE TIME OF ATTACHMENT.[1]

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Knowledge of the actual process of attachment and of the reaction of the organism immediately preceding this act is very meager for many of the sessile marine organisms. As a large number of these forms cause fouling on ship's bottoms, a careful study of some has been made by the author as part of a study to determine the nature and extent of fouling on ship's bottoms.

The activities of the tadpole larvæ of several species of tunicates have been carefully studied by Caswell Grave (1921–1923). He found that after a free-swimming period which varied from a few minutes to several hours, the larvæ invariably became negative to light and attached by means of adhesive papillæ. The factors which determined when the organisms attached appeared to be related to the physiological state of the organism, while the place of attachment is probably a matter of chance, although the reactions to shadows may determine this in some degree.

Nelson (1924) found "that in the oyster the larvæ 'test out,' by means of their feet, an appreciable area of solid surface before actually attaching, which is preceded by circling movements." It had long been supposed that these larvæ had simply settled and the lucky ones survived.

The only record of attachment of larval barnacles of which the author is aware is found in Darwin (1851). He states that "the larva fixes itself with its sternal surface parallel and close to the surface of attachment, and the antennæ become cemented to it; if the cirripede, after its metamorphosis had remained in this position, the cirri could not have been exserted, or only against the surface of attachment; but there is a special provision that the young cirripede shall immediately assume its proper position at

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right angles to the position which it held whilst within the larva, namely with its posterior end upwards. This is effected in a singular manner by the exuviation of the great compound eyes, which we have seen are fastened to the outer arms of the double UU-like, sternal apodemes; these together with the eyes stretch transversely across, and internally far up into the body of the larva; and, as the whole has to be rejected or moulted, the membrane of the peduncle of the young cirripede has necessarily to be formed with a wide and deep inward fold, extending transversely across it; this when stretched open, after the exuviation of the larval carapace and apodemes, necessarily cause the sternal side of the peduncle to be longer than the dorsal, and, as a consequence, gives to the young cirripede its normal position, at right angles to that of the larva when first attached."

Barnacles attach to a great many materials and under very diverse environmental conditions. Darwin (1853) stated that " sessile cirripedes adhere to all sorts of objects, floating and fixed, animal and vegetable, living and dead, organic and inorganic," and that " living mollusca are the most frequent objects of attachment." The following list of materials will serve to give some idea of the varied array of materials on which the writer has seen barnacles attached: wood, glass, rubber, beeswax, shells of more than thirty varieties, rocks of many sorts, rope, cloth, leather, metals of many kinds including iron, copper, zinc, lead, tin, aluminum, and to many alloys as brass and bronze, turtles, crabs, lobsters, fucus fronds, bamboo, zostera, sponges, corals, echinoderms, bryozoa, tunicates, and several types of cetacea. It is thus apparent that barnacles attach to almost every type of material submerged in sea-water, which is sufficiently large and permanent to afford a place of growth.

But although barnacles attach to all these materials, there is nevertheless a high degree of specialization among them. Thus *Balanus galeatus* is found only on certain gorgonian corals; *Coronula diadema* only on the humpback whales (*Megaptera*); *Cryptolepas rachianecti* only on the Californian grey whale (*Rhachianectis glaucus*). *Octolasmis mulleri* is found in the gill chambers of several crabs, while *Octolasmis geryonphila* is found only in the gill chamber of a single type of crab (*Geryon quin-*

quedens). *Chelonibia patula* grows only on the carapace of certain crabs (and on *Limulus*) while *Chelonibia testubinaria* grows only on the carapaces of turtles.

Although many thus show a high degree of specialization as regards their habitat, yet within a single genus one often finds wide extremes. *Balanus amphitrite* is perhaps the most widely distributed barnacle existing today, with a world wide distribution, being found in all tropical and warm temperate waters. It attaches to wood, to rocks, to fucus fronds, to rubber, to metals of many sorts and to shells as well as living molluscs of many kinds. In contrast to this, *Balanus galeatus* attaches only to a limited number of gorgonian corals and is typically a West Indian species, although a few specimens have recently been found from Southern California.

The larvæ of all barnacles are hatched as nauplei, the eggs being carried within the mantle cavity of the adult until hatched. These nauplei moult from one to three times during the course of the first week or ten days, changing slightly with each moult into forms known as metanauplei and then metamorphose into an entirely different type of organism called the cypris larvæ or cyprid. The cyprids of all barnacles are bilaterally symmetrical organisms about four times as long as high and often ten times as long as broad. These proportions vary depending upon the genus of the barnacles but the species of any given genus seem to vary but little in this regard.

This free-swimming period as cyprids appears to vary greatly with different forms of barnacles. *Balanus amphitrite* has been observed to attach within seven days after hatching, while *Chthamalus fragilis* probably attaches within four days. *Balanus balanoides,* on the other hand, has a much more extended larval life and apparently does not attach for from 10 to 12 weeks after liberation.

These cyprid larvæ propel themselves through water by means of sudden backward movements of their appendages. These movements occur with great rapidity so that it is quite impossible to see this reaction unless the organisms are swimming very slowly.

They are aided during their pelagic life by the fact that they possess great globules of a fatty substance in the anterior portion

of their bodies. That this is clearly a lipoid in its character has been demonstrated by its reaction with Congo red and with osmic acid. During much of this period this mass of liquid substance seems to act as a buoy holding the larvæ near the surface film. But as the free swimming period draws to a close this substance disappears. It seems probable accordingly that the length of the free-swimming period is dependent upon the amount of this substance originally stored and its rate of disappearance.

Throughout this period of pelagic life the cyprids often swim on their sides, that is, with their oral surfaces on a horizontal plane with their dorsal surfaces. This holds good especially if the organisms are in the surface film at the surface of the water or if they rest on some object at the bottom. They will frequently propel themselves for some time through a considerable distance while in this position. It has also been observed that they usually are found when in this position to have their morphologically right side turned downward while their left side is uppermost.

The actual process of attachment occurs, as suggested above, after a free-swimming period of from three days to two weeks (longer for some species). The cyprids of *Balanus improvisus* and *Balanus amphitrite* have been kept under observation for ten or eleven days at the end of which time some were found to have attached but many were still active.

When the internal physiological conditions necessary for attachment are present, conditions apparently correlated with the "lipoid" content of the organisms, the larvæ have been observed on many occasions to "walk" on the substratum apparently "hunting" a place for attachment. This remarkable performance is accomplished by alternate attachment and release of the adhesive tips of the antennæ combined with a "push" from the set of appendages.

By reference to Fig. 1, it will be seen that when one of the antennæ (a–2) is released the appendages (ap) straighten out, throwing the larva forward. The released antenna (a–2) then attaches, while the appendages are withdrawn, and with the next move the antenna (a–1) is released.

In this manner these organisms have been observed to traverse a distance of more than twelve mm. (about ½ inch) and have been

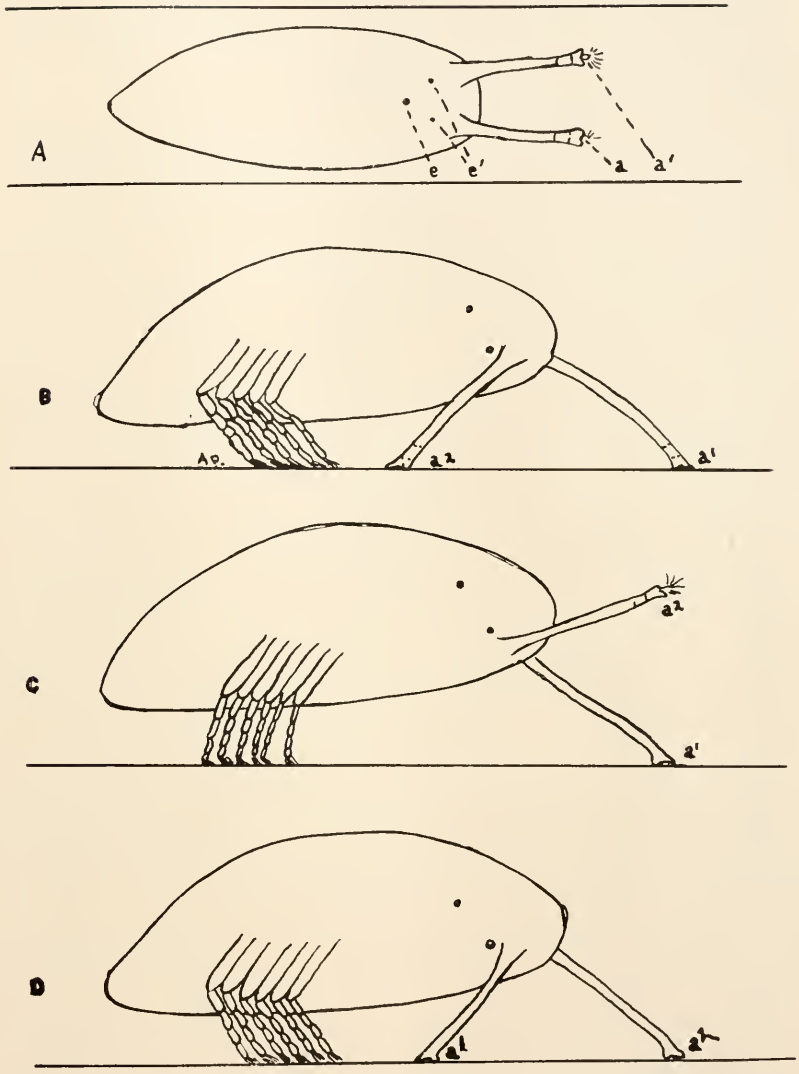

FIG. 1. To illustrate the movements of barnacle larvæ at the time of attaching. *A:* ventral view to show antennæ, a' and a^1; median eye, *e*, and the paired lateral eyes, *e'*. *B–D*, lateral views of cyprid larva, showing method of locomotion at time of "selecting" a place for attachment.

seen to "test" different areas for a period of more than one hour before finally attaching. Frequently it has been observed that the antennæ appear to adhere so firmly that the larva "kicks" for eight or ten times before releasing its hold.

On several occasions the writer has been fortunate in seeing the actual process of metamorphosis while observing through a microscope giving a magnification of about one hundred and fifty diameters. It was then seen that after attachment by means of the antennæ, the organisms "kick vigorously" for some time, but without effecting release. The animal then appears to settle down as soon as attachment was effected, in the region of the appendages. Metamorphosis now occurred. The two-valved shell of the cyprid stage was thrown off, including the exoskeleton of the appendages (often also the paired eyes). From the resulting almost amorphous mass the young barnacle is soon made out. A secretion is continually laid down on the formerly ventral surface, and the rudiments of a coating (the future shell) appear around the sides of the mass. After one hour the "head" and appendages of the animal can be seen in an inverted condition. Whereas when attached the appendages extend downward, they now extend upward and the mouth parts too have changed their position; this reversal in position occurring as described by Darwin as quoted above.

No opercular valves appear until after six hours, while the separate plates of the shell do not appear until more than twelve hours after metamorphosis, in any of the observed cases of *Bal. amphitrite, Bal. improvisus,* or *Chthamalus fragilis.*

In these types the plates appear as four units taking the place of the original apparently chitinous material which protected the organism during the early stages of metamorphosis.

The cyprid larvæ of barnacles are also sensitive to light and it has been shown (Visscher and Luce, 1928) that they are stimulated to a much greater degree by a given amount of energy in the field of green than by like amounts of energy in other fields of the spectrum. These larvæ are usually positive in their reactions but in the later stages they are very erratic and may not show either positive or negative reactions for a considerable period of time. However, at the time of attachment, although still sensitive to

light and especially to wave-lengths of 470–500 $\mu\mu$ they become decidedly negative and move away from the source of stimulation.

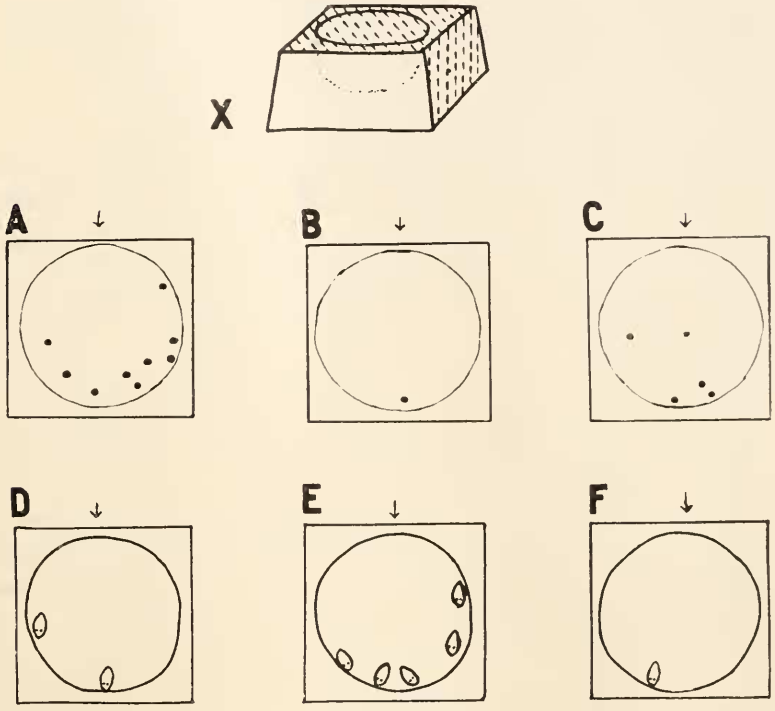

FIG. 2. To illustrate effect of light at time of attachment of larval barnacles. *X*, glass dish, 4 cm. square, by 1.5 cm. in height, with concave depression 2½ cm. in diameter and 1 cm. in depth, covered with heavy black paper on five of its six sides as indicated. *A–C*, and *D–F*, two series of tests, contained barnacle larvæ, in which each container was covered as in *X*, and with the exposed side toward the window as indicated by the arrow. Only those larvæ which had attached and metamorphosed are indicated.

It has been impossible to select cyprid larvæ of known age, but the following experiment will demonstrate this reaction. A number of cyprid larvæ were isolated from a collection of tow made in the tidal channel in front of the laboratory island. From the original lot those with the least amount of "oil droplets" were selected and placed in small glass dishes resembling salt cellars which were surrounded on five of the six sides with a dull black paper (Fig. 2). These were then set on a table in front of a large

north window with the uncovered side of the dish toward the window.

In the first series of three experiments twelve cyprid larvæ of *Bal. improvisus* were placed in *A*, three cyprids of *Chthamalus fragilis* were placed in *B*, and between twenty and thirty mixed forms were placed in *C*.

By referring to Fig. 2, it will be seen that attachment occurred almost without exception on the side of the dish away from the source of light. It was observed at this time that many of these young barnacles were definitely oriented with their anterior ends (containing their eyes) decidedly away from the light side of the dish. In a second series of experiments *D*, *E*, and *F* (Fig. 2) this feature is even more clearly seen. Although these experiments were repeated some two weeks later with large numbers of individuals only a very few were observed to attach, and the results were no more conclusive than those shown in the first two series of experiments.

It can be clearly seen from these experiments that for the two types of barnacles which were tested, light is an important factor in determining the point of attachment, and that they orient with the anterior end directed away from the source of light.

Conclusions.

Barnacle larvæ are sensitive to light at the time of attachment and for the three species tested, are negative to light at this time. It has also been demonstrated that barnacle larvæ do not attach at random, *i.e.*, merely by chance, but that they apparently "test out" the surface before attaching, in which process they have been observed to spend a very considerable period. The antennæ are evidently the "feelers" as well as the final "hold-fasts" and once definitely attached a secretion is deposited which protects the young barnacle from the beginning of its life as a sessile organism.

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REACTIONS OF THE CYPRID LARVÆ OF BARNACLES TO LIGHT WITH SPECIAL REFERENCE TO SPECTRAL COLORS.[1]

J. PAUL VISSCHER AND ROBERT H. LUCE.

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INTRODUCTION.

It is well known that the larvæ of barnacles as well as the larvæ of almost all sessile marine organisms are, at times, sensitive to light and are either photopositive or photonegative. This fact has led to their use for experimental purposes, but the use of the larval forms of barnacles in such experiments has been confined to the earliest free swimming stage call the *Nauplius*. No records have been found of the use of the later larval stage, the cyprid larva in experiments with light.

Since barnacles are the most important of the various types of organisms which cause fouling (cf. Visscher, 1928) and since barnacles attach while in the cyprid stage and subsequently metamorphose into the adult form and since it had been observed on ship's bottoms that barnacles were frequently most numerous on the shaded area of the hull, it seemed important to ascertain the stimulating efficiency of light of different wave-lengths in regard to the cyprid larvæ of barnacles.

In previous work on the reactions of various organisms to color, much confusion has arisen from the fact that the apparatus used was neither standardized nor calibrated. Where spectral transmissions were known, there was frequently no attempt to balance

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the radiant energies. The only occurrences of the use of different spectral colors with balanced radiant energies are those of Pergens (1899), Blauuw (1908), Laurens (1911), Day (1911), and Gross (1913).

The most common error in the earlier work was that of ascribing the effect of colored light to its wave-length, and ignoring the effect of intensity. Pergens was the first to recognize the difference between wave-length and intensity. Working with a spectrometer he found that the energy of the blue end of the spectrum was too small to be measured. He then used light filters of colored glass, and measured the light intensity with a Ritchie photometer. Pergens failed, however, to eliminate the effect of the infra red transmitted by the filters.

Laurens, Day and Gross used apparatus which was essentially alike. The light was diffracted and balanced and the band of transmission was either narrowed or widened until the deflection of the galvanometer was the same for all the colors. While this work was done with colors of high purity and balanced radiant energies, it cannot give more than a relative idea as to the stimulating efficiency of parts of the spectrum, as only four limited regions were employed.

Mast (1917) used a Hilger spectrometer and a Lummer-Brodhun rotating sector and was able to determine with great accuracy the relative stimulating efficiency of the various parts of the spectrum. While this method allows a direct study of the effectiveness of spectral colors, it appeared to be limited in its application to organisms which, in a single beam of light travel parallel to the rays and do not deflect to either side and consequently could not be used with cyprid larvæ. The intensity of light obtainable by this method was believed to be too limited for adequate work on the cyprid larvæ of barnacles. For these reasons and others, to be discussed later, it was considered inadvisable to use this method in the following work.

Materials and Methods.

The larval life of the barnacle is divided, with a very few exceptions, into two distinct stages, the nauplius, and the cyprid. The cyprid stage is characterized by the presence of a large bi-

valve shell, giving it a general resemblance to certain ostracods. This resemblance, however, is superficial and does not extend to the limb structure or internal anatomy. All of the appendages of the adult are present; the antennules protrude from between the valves of the carapace; there are six pairs of thoracic limbs, but no abdominal appendages. The unpaired eye of the nauplius is usually still present, and in addition there is a pair of large compound eyes.

Cyprids of various species of barnacles differ from each other in the size and proportions of the carapace, and in the presence of certain characteristic bodies within the carapace. During the time of year in which this work was done, three main types of cyprids predominated. These types were of two distinct sizes. The smaller size was .51 mm. long, .225 mm. high, and .20 mm. wide, and was found by subsequent metamorphosis to be the cyprid of *Chthamalus fragilis*. The larger type was made up of two groups which were outwardly very similar, being .65 mm. long, .24 mm. high, and .20 mm. wide. These groups could only be distinguished by the presence of certain granules in the carapace. One of these was determined by subsequent metamorphosis to be *Balanus improvisus*, and the second was the cyprid of *B. amphitrite*.

Balanus improvisus and *B. amphitrite* are known to breed most abundantly at Beaufort during June and July, and large numbers of their nauplii and cyprids are to be found at that time. *Chthamalus fragilis* has been observed to have a more extended breeding period, apparently running from May into late September. *Balanus eburneus* is abundant at Beaufort, but as far as is known, no cyprids of this species were used in the experiments.

Cyprids were obtained by towing with a number 12 mesh net. They were picked out of the tow by means of a capillary pipette, then washed in clear sea water and placed in the aquarium. During the summer of 1924 cyprids were not numerous but during the summer of 1925 cyprids were very abundant, comprising at times in late June and early July a large part of the plankton. This abundance allowed a selection of material, and in consequence most of the work was carried out with the cyprids of *B. improvisus* and *B. amphitrite*. Cyprids appear to be most abundant

on an incoming tide and at flood tide. This is also true for the nauplii during June when they were most abundant. There is a marked diurnal migration, so that tows during the day must be made with the net about eight feet below the surface, this being slightly less than the minimum depth of the channel. The time of exposure to sunlight exerts a marked influence on the activity of those cyprids which remain at the surface. Cyprids taken from a surface tow during the morning are fairly active, but those taken from a surface tow during the late afternoon are inactive, and could not be used.

The apparatus used in the following experiments consisted of 100 watt, gas-filled, tungsten lamps, two copper sulphate cells, and thirteen monochromatic light filters.

In selecting the filters, an attempt was made to use only those having a narrow transmission band. This is somewhat difficult to obtain for the yellow and blue-violet. Wratten filters were available for most of the needed colors, but these filters are not wholly stable under the climatic conditions found at Beaufort. At the suggestion of Dr. K. S. Gibson of the Bureau of Standards, we were able to obtain a number of filters from glasses manufactured by the Corning Glass Works, which company very kindly supplied them for our use. These glasses were ground to thicknesses giving transmissions in accordance with the curves shown in Bureau of Standard Technologic Paper 148. The glasses are more satisfactory than Wratten filters because of their permanency under all climatic conditions. However, Corning glasses were not available for certain regions of the spectrum, so four Wratten filters were used, making a series of thirteen filters covering the whole visible spectrum. By referring to figure No. 1 a list of all "Corning" filters with their curves of transmission can be found. Table I. is a summarization of the curves for all filters used giving the limits of transmission and the dominant wave-length of each filter.

The difficulty encountered in obtaining a deep blue or ultraviolet arises from the fact that all the filters in this region transmit some of the red end of the spectrum. No filter was available to block out the red without also cutting off some of the blue or ultraviolet. Since red is relatively feeble in stimulating effect, it

was judged best to use the blue and untraviolet filters as they were, and to attempt a correction for the presence of the red later. We were not able to obtain a yellow filter with a sharply limited transmission band, since ordinary yellow filters merely cut off a portion of the blue end of the spectrum. Perhaps the best yellow would be produced by a combination of Corning glasses G36

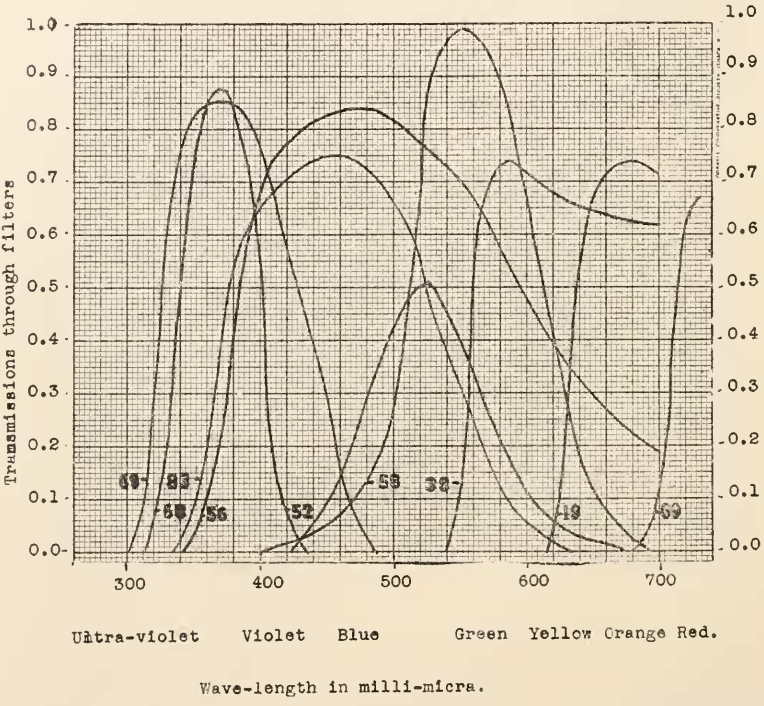

FIG. 1. Curves to illustrate the range of light energy transmitted by the "Corning" glass filters used to test the relative stimulating efficiency of spectral colors. (From the U. S. Bureau of Standards Technologic Papers No. 148.)

(curve 38) and 401Z (curve 59) as given in Bureau Standards Paper No. 148. Unfortunately, however, this combination was not available to us at the time. The balancing of the radiant energies for all filters was done by the Bureau of Standards, by the use of an apparatus based upon the radiomicrometer of Boys.[1]

[1] The authors are grateful to the Director of the Bureau of Standards and also to Dr. W. W. Coblentz for their kindness in calibrating all filters and lamps used for these experiments.

Table I.

List of Filters Used in Experiments, Showing their Total Spectral Transmission, and their Dominant Wave-Lengths.

Filter.	Total Transmission	Dominant Wave Length.
Ultra C 83	315–428 μμ 609–Red End	355 μμ
Purple C 69	310–485 μμ 690–Red End	370 μμ
Purple W 35	300–475 μμ 650–700μμ	420 μμ
Blue W 49	400–510 μμ	440 μμ
Blue C 60	335–640 μμ	460 μμ
Blue C 59	335–690 μμ	480 μμ
Blue Green C 56	340–700 μμ	505 μμ
Green C 52	425–670 μμ	530 μμ
Green W 58	485–635 μμ	540 μμ
Yellow W 15	500–700 μμ	590 μμ
Orange W 22	545–700 μμ	620 μμ
Orange C 38	540–Red End	640 μμ
Red C 19	620–Red End	700 μμ

The letter "C" after a filter denotes a Corning glass filter. The numbers after the Corning glasses refer to the transmission curves shown in Bureau of Standards Technologic Paper 148. The letter "W" denotes a Wratten filter, and the number refers to the transmission curves found in the booklet "Wratten Filters" published by the Eastman Kodak Company.

Each lamp to be calibrated was set up with the center of the curved filament facing a radiometer. The radiometer was placed on a scale graduated in centimeters, and could be moved toward or away from the light. Connected with the radiometer was a Coblentz modification of Thompson's galvanometer. This was hung on the wall, fifteen feet from the radiometer, and at right angles to it. The lamps used were 100 watt, 115 volt, gas-filled, Mazda lamps and during the test each lamp carried 109.3 volts and 0.87 amperes. In these experiments the infra-red was absorbed by copper sulphate cells. Each cell was two centimeters thick, and consisted of two pieces of optical glass cemented to a glass ring, five centimeters in diameter. The cells were filled with a solution consisting of 57 grams $CuSO_4$ in two liters of water. One of these cells was placed in front of each lamp tested. The radiometer was then moved back and forth until the galvanometer showed a deflection of one centimeter. This was taken as a

standard deflection. A filter was then placed before the lamp and the galvanometer again brought to a deflection of one cm. Such balancing of the radiant energies, therefore, eliminates the necessity of correcting for the unequal distribution of energy in the spectrum of the lamp. The result of any experiment conducted with these balanced filters represents, therefore, the actual stimulating efficiency of the specific wave-length of the light, and is not in any way influenced by a difference in light intensities.

The apparatus was set up so that two beams of light crossed at right angles (Fig. 2). The lamps were enclosed in light-proof boxes each having an opening five centimeters square. A copper sulphate cell was placed in front of each of these openings. Each box was readily moved, and the center of the crossing of the two beams was used as the zero point from which to measure the position of the light. The aquarium in which the organisms were placed was made of the best quality slide-glass, and was 35 mm. square and 10 mm. deep. This aquarium was placed on a stand at the center of the two cross beams. Underneath this aquarium was placed a small microscope lamp which could be lighted momentarily so that the cyprids could be counted more readily. Two screens were placed just outside of the aquarium stand, one of these had an opening admitting white light, and the other had a slot in which the filters were placed. In practice, the lamp giving the white beam was placed permanently with the center of the filament at a point 119.2 cm. distant from the center of the two beams of light while the lamp used with the filters was moved to such distances as to compensate for the energy transmission value as previously determined for each filter.

Ten cyprids were placed in each aquarium in carrying out an experiment. They were allowed to become dark adapted, and were then given a one minute exposure to light. At the end of this time the cyprids in the white light, in the filter light, and in the corner between the two light beams, were counted. These numbers thus obtained were recorded in a table of four columns under the heads of White, Corner, Filter, and Non-reacting. A dark period of about twenty seconds was given between trials. Three trials were made with each filter, after which the filters

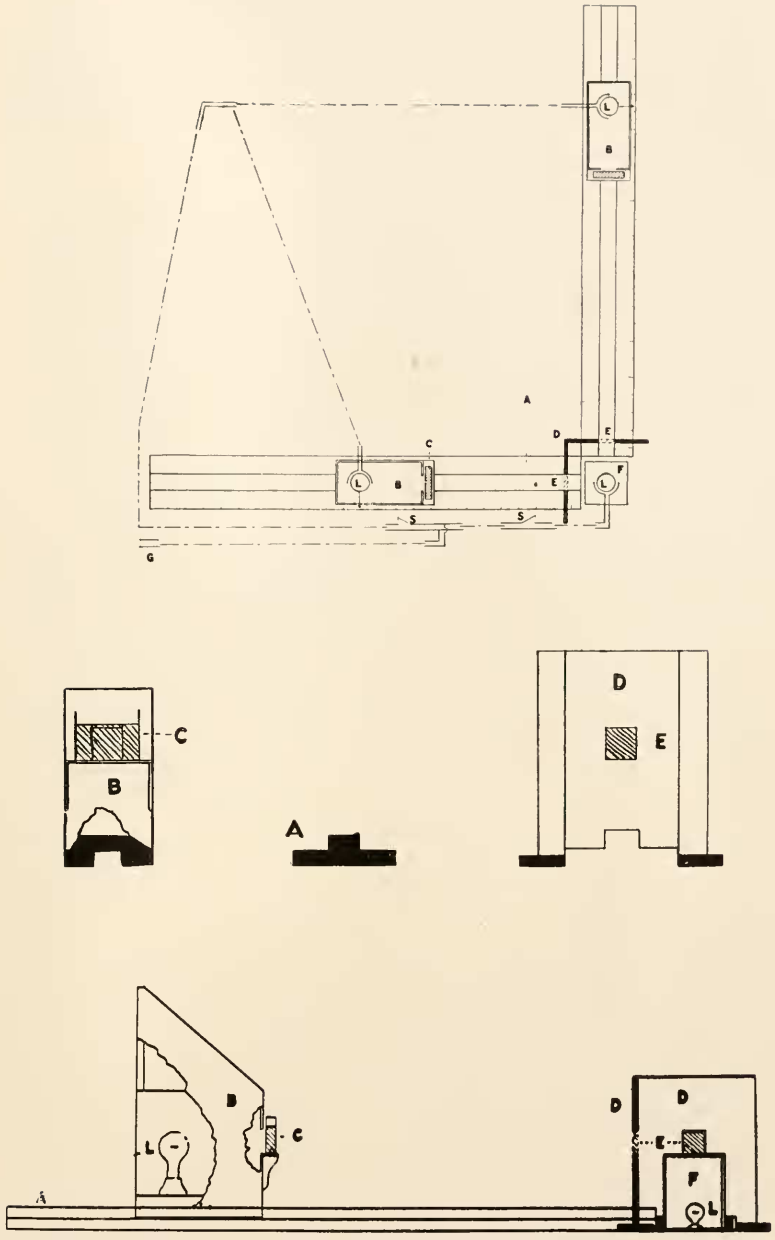

FIG. 2. To illustrate apparatus used to test the relative stimulating efficiency of equal amounts of light energy, in known and limited fields of the spectrum, upon the cyprid larvæ of barnacles. *A*, Track for boxes (*B*) containing the electric bulbs (*L*). *C*, copper sulphate filters in front of square opening in light box (*B*). *D*, screen holding filters (*E*) and surrounding aquarium stand (*F*). *S*, switches for control of lights. *G*, feed line for electric current.

were changed. The same cyprids as a rule could be used for the whole series of filters, though certain factors modified their behavior.

Behavior of Cyprids.

Cyprids give definite reactions to light for two to three hours, but after that the number of non-reactants increases rapidly. They must also be dark adapted in order to secure definite movements toward the light, but apparently there are limits to this dark adaptation. It was noted during the experiments that if the cyprids were left in total darkness for periods of thirty to sixty minutes, that on resuming the experiments there were no reactants during the first exposure. In the second exposure there were a few reactants, but several exposures were required before the usual number of reactants were obtained. This, however, can be demonstrated more strikingly with nauplii, where, in several instances, ten exposures were required before all of the nauplii were reacting. The effect of a shorter dark period is also well marked. A dark period of one minute was used during the earlier experiments. By varying this rest period, however, and counting the number of reactants and non-reactants, it was found that a rest period of about twenty seconds gave the smallest number of non-reactants. Such a period varies somewhat as the experiment progresses, for a shorter period can be used in the first part of the experiment, and a slightly longer period is necessary toward the end.

Cyprids usually lie upon either side when not in motion. Movement is usually on the side, and is brought about by rapid and powerful kicks of the thoracic appendages. The movement is rapid and usually in a wide arc, but sometimes in a straight line. It is not at all unusual for them to turn about in a complete circle or series of circles before shooting off toward the light. The position of the antennæ apparently has much to do in determining the direction of movement, the movement being in a wide arc or fairly straight line when the antennæ are withdrawn into the carapace, and circular when they are extended. The cyprids may either sink down and lie quietly on the bottom after striking the side of the aquarium, or they may move back and forth along the side of

the dish in a series of jerky movements. This uncertainty in their movements makes it necessary to have a definite time of exposure, and also necessitates a rapid counting of those collected in each light.

The presence of small oil droplets in the anterior end of the cyprids modifies their behavior to a considerable extent. These droplets are usually present, and are found either in the anterior end or along the dorsal side. Their size may apparently be taken as an indication of the age of the cyprid. Cyprids about to attach have very small drops, are sluggish, and move about on the bottom of the dish with a walking movement of the antennæ and a push or slow kick of the thoracic appendages. Those in which the oil droplets are large, often become caught in the surface film. When this occurs it is difficult to get them out, and while they may be very active, they cannot react definitely toward the light. Their reaction consists of spasmodic kicks and short movements toward the light, but these movements soon cease and they drop back passively toward the center of the dish. If two or more cyprids get into the surface film, they cluster together and are of no further value in the experiment. In such cases they were removed and replaced by fresh organisms. The cyprids which are the most favorable for study are those which are too old to get in the surface film, and which are not ready for attachment.

Results.

In Table II. are shown the results of twenty series of experiments as described above. These results are summarized in figure 3 showing a curve which clearly indicates that there is a fairly wide region in the spectrum from 505 to 590 $\mu\mu$ or from light blue to yellow, where the stimulating efficiency is equal to, or more than 50 per cent. of white light. The maximum of this region is in the light green from 530 to 545 $\mu\mu$ where the stimulating efficiency is between 90 and 94 per cent., or practically the equivalent of white light.

In the region of the spectrum from the yellow-orange through the red end, or from 590 to 700 $\mu\mu$, there is a very sharp drop in the effectiveness of light, until at 700 $\mu\mu$ this is less than 5 per cent.

of white light. On the other side of the maximum there is a fall in value from 480 to 440 $\mu\mu$, but this is not as sharp as on the red end. From 420 into the ultra-violet there is a flattening of the curve which indicates strongly that ultra-violet light has a distinct stimulating effect. This effect, however, is very limited in comparison with the maximum in the field of green.

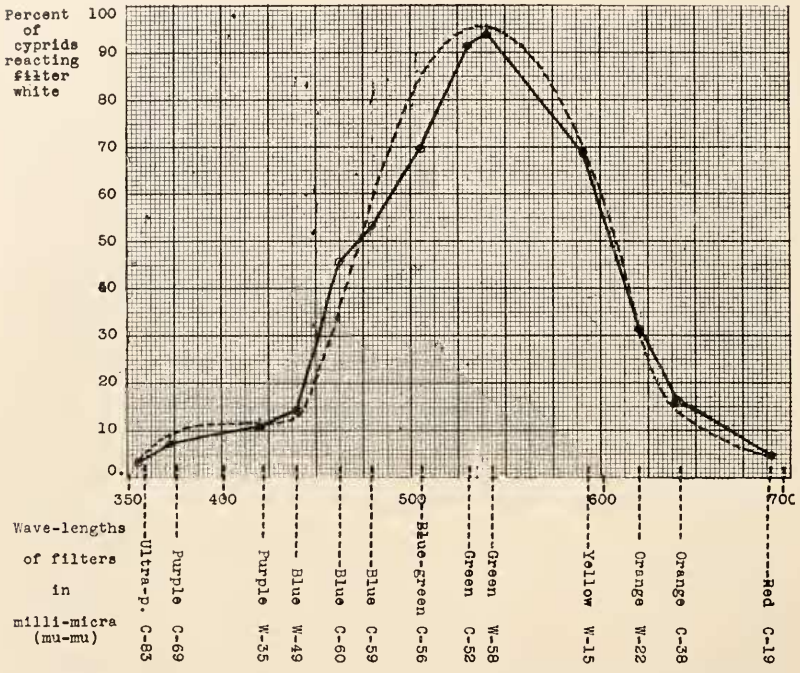

FIG. 3. A curve showing the distribution of the stimulating efficiency of equal energy values among the various parts of the spectrum, for the cyprid larvæ of *Balanus amphitrite* and *Balanus improvisus*.

Due to the fact that light from a tungsten filament does not penetrate far into the ultra-violet, we were unable to test this region as carefully as desired, but in all tests the relative energy values were carefully calculated.

No filter transmitting ultra-violet was available which did not at the same time transmit some light in the far-red end of the spectrum. (Compare No. 69 in Fig. 1.) The efficiency values for these filters were therefore corrected for the presence of red by

Table II.

Results of Twenty Sets of Experiments to Determine Relative Effects of Equal Light Energies in Different Fields of the Spectrum, upon the Cyprid Larvæ of Barnacles.

Number of Filter	Ultra C 83		Purple C 69		Purple W 35		Blue W 49		Blue C 60		Blue C 59		Blue C 56		Green C 52		Green W 58		Yellow W 15		Orange W 22		Orange C 38		Red C 19	
Dominant Wave Length	355 μμ		370 μμ		420 μμ		440 μμ		460 μμ		480 μμ		505 μμ		530 μμ		540 μμ		590 μμ		620 μμ		640 μμ		690 μμ	
Limits of Transmission	315–428 μμ 690– Red End		310–485 μμ 690– Red End		300–475 μμ 650–700 μμ		400–510 μμ		335–640 μμ		335–690 μμ		340–700 μμ		425–670 μμ		485–635 μμ		500–700 μμ		545–700 μμ		540– Red End		620– Red End	
Number of Trial.	White	Filter	White	Filter	White	Filter	White	Filter	White	Filter	White	Filter	White	Filter	White	Filter	White	Filter	White	Filter	White	Filter	White	Filter	White	Filter
1	27	3	25	3	28	2	30	3	20	7	28	13	19	10	14	13	14	15	13	11	20	12	24	6	31	4
2	28	2	27	5	26	2	25	5	14	10	30	10	19	12	19	16	20	15	24	14	20	9	28	11	32	4
3	33	1	28	1	28	2	30	4	19	7	18	12	16	11	21	19	18	15	19	15	19	7	29	7	34	1
4	30	3	30	3	32	2	24	3	18	10	21	11	19	12	17	16	19	14	23	16	18	8	23	3	33	2
5	26	1	24	2	26	4	23	2	14	9	13	10	14	10	18	17	19	15	21	13	26	7	26	6	32	2
6	29	1	27	2	27	4	24	3	14	6	16	7	15	11	13	10	19	15	23	15	25	6	24	4	27	2
7	22	2	27	1	27	4	28	5	23	9	23	10	22	14	16	22	16	15	22	18	25	6	33	6	35	1
8	36	2	32	3	26	3	32	4	24	9	20	10	13	11	12	15	17	16	20	13	29	5	24	3	26	1
9	20	1	18	2	28	3	22	4	18	6	17	14	15	13	15	16	19	17	19	11	26	5	30	5	33	1
10	32	2	33	4	25	3	29	4	20	14	18	12	16	12	14	15	17	14	19	14	24	6	22	2	32	1
11	32	1	30	1	25	4	32	5	19	10	18	14	21	15	21	19	11	15	17	9	20	7	24	3	25	2
12	35	1	32	3	22	4	33	2	22	9	24	12	21	15	20	15	16	10	20	11	24	7	26	2	30	1
13	29	2	28	2	27	4	28	3	23	8	22	10	21	15	18	15	18	14	17	13	23	8	25	5	30	0
14	30	1	27	6	29	3	32	3	20	14	15	8	20	10	22	17	17	19	15	10	22	6	29	3	29	1
15	36	0	32	2	25	5	32	3	20	8	20	7	18	15	18	18	15	15	22	15	18	6	29	4	37	1
16	34	1	31	2	25	4	30	5	23	13	21	12	24	17	21	17	12	11	20	14	22	7	24	3	29	2
17	34	1	33	1	26	4	28	4	30	5	24	8	26	10	20	18	12	14	19	14	21	4	30	4	31	1
18	35	2	27	4	26	2	27	6	20	11	21	13	19	14	20	17	13	19	21	13	21	8	27	3	31	0
19	25	1	32	2	23	4	31	4	25	13	23	16	25	16	20	17	12	20	14	12	24	8	27	5	30	1
20	30	0	29	2	27	3	26	3	25	10	28	15	18	12	18	15	13	12	19	15	20	9	33	3	35	0
Total number of reactants to either light	603	28	572	51	528	66	534	75	411	188	420	224	381	265	357	327	319	300	387	266	455	141	537	88	622	28
Per cent. of stimulating efficiency as determined by dividing the number reacting to white with the number reacting to filter	4.6% Corrected for Red 3.2%		8.9% Corrected for Red = 7.4%		12.5% Corrected for Red = 10.9%		14.2%		45.8%		53.4%		69.6%		91.6%		94.0%		68.7%		31.1%		16.4%		4.5%	

determining the percentage of red transmitted and then deducting that value from the total value on the basis of the effectiveness of that region in the red.

During the course of this work on cyprids it was also found possible to test the reactions of the nauplii of four species of barnacles to these lights as described above. These results were uniform in that the region of the spectrum between 530 and 545 $\mu\mu$ was in each case the most stimulative. This maximum stimulating effect was the same both when these organisms were negative and when positive to light.

It is of interest that our results are in substantial agreement with the work of other investigators using nauplii of barnacles: Loeb & Maxwell, 1910, Loeb & Westeneys, 1916 and Loeb & Northrup, 1917. Thus it is apparent that the same region of the spectrum offers the greatest stimulating efficiency in both the nauplius and in the cyprid which is not surprising in view of the fact that the nauplius eye is still present in the cyprid.

Conclusions.

The cyprid larvæ of barnacles agree with the larvæ of most other marine organisms in that they react to light. With equal radiant energies and with the infra-red heat rays eliminated, a distinct difference in stimulating effectiveness is found between different regions of the spectrum. This difference shows, for the cyprids of the two species of barnacles tested, a region of maximum efficiency in the light green, between 530 and 545 $\mu\mu$. It is of interest to note that similar results have been found for the larvæ of other marine organism. The larvæ of the hydroid, *Eudendrium* are most sentitive to blue light (460–480 $\mu\mu$); the larvæ of *Arenicola,* to the green (about 495 $\mu\mu$); and the larvæ of the squid. to blue green (470–510 $\mu\mu$).

However from the results obtained by Hess (1910), Mast (1917) and Hecht (1921), it is apparent that the distribution of stimulating efficiency in the spectrum is not necessarily the same for all organisms, which react to light. It is consequently apparent that there is no true color vision in these lower forms—in the usual interpretation of the term, but rather that the photo-sensitive materials in the light perceiving organs are more sensitive to the

effects of certain wave-lengths than to others. Increased intensity of other wave lengths may however produce similar results.

This investigation has, however, an important bearing on the problem of the nature and extent of the fouling of ships' bottoms, an intensive study of which is being completed by the senior author and of which this work forms a part. It has been found that light is an important factor governing the attachment of the organisms which cause fouling and that at the time of attachment many of these larvæ are negative to light, for they are found most abundantly on the shaded portions of the ships' hull.

Since barnacles are the most important factor in fouling it seems probable that the studies reported above may have some significance in the solution of this problem, for if a light green paint were used in place of the red, now universally used, a considerable benefit should be derived, on theoretical grounds, provided all other factors were comparable.

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VITALITY OF THE GAMETES OF *CUMINGIA TELLINOIDES.*

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Cumingia tellinoides is a small lamellibranch mollusk found abundantly in the Woods Hole region. Its breeding season extends over a period of three or three and a half months, beginning early in June. The production of eggs is continuous and spawning by each mature individual occurs two or three times during the course of the breeding season. Eggs may be had in great abundance during this time. One female may shed 300,000 eggs, although the average number extruded is estimated to be between 100,000 and 200,000. They are expelled into the sea water where they are fertilized.

The present paper is a report on experiments to determine the vitality of the gametes, and more especially the longevity of the unfertilized eggs. It may be assumed that when eggs are released from the ovary, they are normal. From the time of liberation, deterioration begins and if not fertilized they ultimately go to pieces by fragmenting in a characteristic manner. The extensive work of Goldforb indicates that some deterioration takes place in sea urchin eggs while stored in the ducts of the gonad before extrusion. This may also be true of *Cumingia*, since two clear cases of physiologically poor lots of eggs have been found among hundreds of normal ones. Deterioration o both gametes of *Cumingia* and of *Arbacia* is rapid after extrusion into sea water.

Vitality of the Eggs.

The eggs used in these experiments were obtained by isolating sexually mature individuals in small stender dishes filled with sea

water into which the eggs were shed. The eggs, so collected in one dish, may therefore be conveniently referred to in the description of the experiments as a "batch" or a "lot" of eggs. The reference in all cases is to the eggs of a single female.

During the summer of 1926 many batches of eggs were kept without fertilization to test their longevity. During the progress of the experiments a few eggs were taken from the dishes from hour to hour and fertilized as a test of their physiological condition and of the rate of deterioration as indicated by decreasing percentage of fertilization. The experiments reported here deal principally with the longevity of the unfertilized eggs as indicated by the time of disintegration.

Approximately two per cent. of the eggs of most females are defective at spawning and fragment within four or five hours. The other ninety-eight per cent. remain intact and capable of development for longer periods. The poorest lot of eggs when tested at a temperature of 20° to 22° centigrade went to pieces completely in six hours, and it was not uncommon for 98 per cent of the eggs of some females to fragment in from eight to nine hours. The great majority of the lots of eggs fragmented in from nine to fifteen hours, the average being between ten and twelve hours. When tested at 18.5° to 20° C., on the other hand, the most vigorous batches of eggs, remained intact from fifteen to twenty hours, that is, the eggs retained their normal appearance for that length of time. It was shown that a majority of the eggs either refuse to develop or develop abnormally, when fertilized shortly before fragmentation. However, a few normal embryos come from the oldest eggs, even when the percentage of fertilization is greatly reduced. When a lot of eggs is fertilized after fifty per cent. of them have fragmented, it not infrequently happens that most of those which remain intact fertilize and cleave. However, most of them die in cleavage or gastrula stages and few develop into normal embryos. The oldest lots of eggs to develop normally were ten and twelve hours old and rarely fifteen hours old. These all showed 95 to 98 per cent. fertilization and normal development.

The extreme limits of longevity of the unfertilized eggs are therefore found to be six to twenty-six hours, and the limits of

fertilization and normal development in the best lots of eggs are ten to fifteen hours. These figures are for temperatures normally experienced by the eggs of the species, or 18.5° to 22° C. They therefore represent the normal longevity of the eggs.

The statement by Morgan that the eggs of *Cumingia* will not stand rough handling is misleading. They are injured by centrifuging but there is abundant evidence that they develop normally under ordinary laboratory manipulation.

Tables I., II. and III. deal with various phases of the longevity and vitality of the eggs and include representative experimental data.

Individual Variation.

After any particular lot of eggs begins to fragment, three or four hours elapse before all or 98 per cent. have fragmented. The variation in longevity within a single group is, therefore, approximately four hours. For example, if 10 per cent. of the eggs have fragmented at twelve hours, the average expectancy of fragmentation for the remainder would be 30 per cent. in thirteen hours, 50 per cent. in fourteen, 70 per cent. in fifteen, and 95 to 98 per cent. in sixteen hours. Two per cent. may remain intact indefinitely. The rate of fragmentation is shown in Table I., as is also an actual comparison of the longevity of five lots of eggs from five females studied under identical conditions. Tables II. and III. show the same thing in slightly different form.

When fresh eggs are fertilized by fresh sperm the percentage of cleavage is usually between 97 and 100 per cent. However, a few lots of eggs gave from 89 to 95 per cent. cleavage. One lot of physiologically poor eggs was observed which when fertilized with physiologically good sperm gave only 20 to 30 per cent. cleavage. This sperm when used to fertilize other lots of eggs gave from 97 to 100 per cent. cleavage showing that failure to cleave on the part of these eggs was not due to defective sperm. This lot of physiologically poor eggs was slimy from the first and showed a tendency to cling to the containing dish. This together with one other lot of defective eggs may be regarded as confirmation of Goldforb's contention that eggs at the time of spawning may show all the symptoms characteristic of aging

Table I.

Vitality of *Cumingia* Eggs.

Record of five lots of eggs spawned by five females on August 16 and kept unfertilized at 20° C. The vertical columns, when read from top to bottom, give the result of eleven examinations that were made with the microscope at approximately hourly intervals in order to ascertain the time that elapsed before fragmentation began in each lot of eggs as well as the rate of fragmentation. The percentages refer to the number of eggs that remain normal in appearance. From three to five hundred eggs were counted in each examination.

	♀ No. 1.	♀ No. 2.	♀ No. 3.	♀ No. 4.	♀ No. 5.
After 5½ hrs.	100% normal	97% normal	100% normal	100% normal	100% normal
After 6½ hrs.	70% normal	100% normal	100% normal	100% normal	100% normal
After 8 hrs.	20% normal	5% normal	100% normal	100% normal	100% normal
After 9 hrs.	0% normal	0% normal	100% normal	100% normal	98% normal
After 10 hrs.			100% normal	100% normal	98% normal
After 11½ hrs.			100% normal	98% normal	90% normal
After 12 hrs.			100% normal	98% normal	80% normal
After 13 hrs.			98% normal	95% normal	50% normal
After 14 hrs.			95% normal	85% normal	30% normal
After 14½ hrs.			90% normal	70% normal	2% normal
After 15 hrs.			80% normal	50% normal	0% normal

Comment.—Eggs from lots 3, 4 and 5 were inseminated with freshly shed sperm after 11½ hours. The percentage fertilization which resulted was estimated to be 90 to 95 per cent. and abundant normal larvæ developed. Many eggs are apparently normal for 12 hours after extrusion. Eggs from lot 3 were fertilized 13 hours after extrusion which gave 75 per cent. cleavage and normal development. It appears that there is great variation in the vitality of the eggs of different females. These five lots spawned by five different females during the same hour were treated alike. One fragmented in seven hours, one in eight hours, one in fourteen hours, one in sixteen hours and it may be predicted in the light of former experience that the other would have fragmented completely in seventeen or eighteen hours if the experiment had been continued. No. 1 began to fragment in six hours. This gives the range of variation in longevity among the eggs from the five females as six to eighteen hours and normal fertilization for twelve hours in the best of the five. These five lots of eggs are substantially typical and may be considered as representative, although some are considerably longer lived than those used in this experiment. At least twenty such experiments were carried out during the season. The extreme limits of longevity were found to be from five to twenty-six hours. Only one or two per cent. of the best lots of eggs remained intact after the expiration of that time. It is interesting to compare this with the egg of *Arbacia* which, according to Goldforb and others, has a longevity of 40 to 48 hours.

TABLE II.

NORMAL VARIATION IN PERCENTAGE CLEAVAGE IN THE EGGS OF *Cumingia tellinoides*.
Temperature 22° C.

August 4. Freshly spawned eggs from ten females were fertilized by the sperm of one male to show the initial variation in the percentage of cleavage of eggs of this species. This table also shows the effect of aging. It is interesting to note that after 90 to 95 per cent. of the eggs have fragmented those remaining intact may fertilize and cleave. (Compare Tables II. and III.) All eggs normal and fragmented, are included in the calculation of the percentages.

	After 2 Hrs.	After 8 Hrs.	After 11 Hrs.	After 14 Hrs.	After 16 Hrs.
Female No. 1.....	98% cleavage	98% cleavage	93% cleavage	10% cleavage	0% cleavage
Female No. 2.....	98% cleavage	98% cleavage	40% cleavage	0% cleavage	0% cleavage
Female No. 3.....	92% cleavage	10% cleavage	0% cleavage	0% cleavage	0% cleavage
Female No. 4.....	97% cleavage	70% cleavage	71% cleavage	50% cleavage	2% cleavage
Female No. 5.....	98% cleavage	7% cleavage	0% cleavage	0% cleavage	0% cleavage
Female No. 6.....	96% cleavage	97% cleavage	92% cleavage	60% cleavage	20% cleavage
Female No. 7.....	99% cleavage	98% cleavage	96% cleavage	2% cleavage	0% cleavage
Female No. 8.....	95% cleavage	95% cleavage	95% cleavage	40% cleavage	10% cleavage
Female No. 9.....	88 to 90% cleavage	30% cleavage	20% cleavage	1% cleavage	0% cleavage
Female No. 10.....	30% cleavage	0% cleavage	0% cleavage	0% cleavage	0% cleavage

eggs. The first vertical column of Table II. shows the usual range of variation in freshly spawned eggs in respect to percentage fertilization and cleavage.

Table III.

Longevity of the Eggs of *Cumingia tellinoides*, Temperature 22° C.

The eggs of the ten females compared in Table II. in respect to percentage fertilization are here compared as to longevity and time of fragmentation or disintegration. It will be noted that the time of reduction in cleavage is almost synchronous with the time of fragmentation. This relationship does not always hold because in numerous experiments the percentage of cleavage fell off before fragmentation. In other words, if the reduction in cleavage due to aging and the rate of fragmentation were plotted as curves they would not as a rule be superimposed or parallel.

	Percentage of Fragmentation with Aging.			
	After 7 Hrs.	After 11 Hrs.	After 15 Hrs.	After 16 Hrs.
Female No. 1.....	0%	0%	50%	100%
Female No. 2.....	0%	45%	100%	100%
Female No. 3.....	75%	100%	100%	100%
Female No. 4.....	0%	2%	72%	90%
Female No. 5.....	73%	100%	100%	100%
Female No. 6.....	0%	0%	40%	50%
Female No. 7.....	0%	0%	99%	100%
Female No. 8.....	0%	0%	12%	60%
Female No. 9.....	0%	7%	100%	100%
Female No. 10.....	100%	100%	100%	100%

Note.—Many of the eggs of lots 4, 6 and 8 lived beyond sixteen hours. The other lots of eggs had fragmented completely after sixteen hours.

Vitality of the Spermatozoa.

The work of Gemmill ('00) and F. R. Lillie ('15) has shown that variations in the concentration of sperm suspensions of *Arbacia* make a great difference in the duration of their fertilizing ability. The writer accordingly used several sperm dilutions in order to learn whether the same phenomena are exhibited by the sperm of *Cumingia*.

Spermatozoa shed by a mature male *Cumingia* of average size in 30 cc. of sea water was estimated by actual measurement to be a 2 to 3 per cent. suspension.[1]

[1] The method of measuring the percentage was to kill the spermatozoa by the addition of formalin and after settling they were measured en masse. From this the calculation of the concentration of the original suspension was a simple matter.

This concentrated suspension, though at best somewhat variable, was used as a standard suspension from which various dilutions were made. It was found possible to select suspensions of approximately the same strength and this is a matter of more importance than that the exact percentage be known.

Two drops[2] of standard sperm suspension when added to 25 cc. of sea water was estimated to be a 1/500 to 1/750 per cent. suspension; two drops in 50 cc. of sea water was considered to be a 1/1000 to 1/1500 per cent. suspension; one drop in 50 cc. of sea water makes a 1/2000 to 1/3000 per cent. suspension, etc. The last named suspension when fresh is adequate to fertilize one hundred per cent. of the eggs whereas greater dilutions sometimes gave only partial fertilization. What was estimated to be a 1/4000 to 1/6000 per cent. suspension gave from eighty to one hundred per cent. fertilization and usually one hundred per cent. All of these suspensions were used and also the same percentage suspensions in larger quantities of sea water.

The method of studying the relative longevity of these various dilute sperm suspensions was to make up, by the proper dilutions, several dishes of each from freshly shed sperm. To these fresh eggs were added in turn at hourly intervals until the suspensions no longer gave fertilizations. It was shown in general that the weakest suspensions die first. Tables 4 and 5 show that the longevity of the spermatozoa depends largely upon the degree of concentration. Even a suspension of 1/500 per cent. shows some preserving effect in that spermatozoa live for a longer time in this concentration than in greater dilutions. There is little difference between a 1/2000 and a 1/6000 per cent. suspension, and these no doubt represent natural conditions so far as longevity is concerned.

In work reported at this time the writer had in mind to study the longevity of gametes under natural conditions. In general the longevity of the sperm in the most dilute suspensions is somewhat less than that of the eggs. In a few cases using suspensions of 1/500 per cent. approximately ninety per cent. normal embryos developed from sperm and eggs that were

[2] The same pipette was used in making all dilutions of an experiment and one giving approximately one cc. per 20 drops

twelve hours old and in numerous other cases at nine and ten hours. As a rule when sperm suspensions of 1/2000 to 1/3000 per cent. are used very few fertilizations occur after nine or ten hours. Numerous experiments show that spermatozoa begin to die after three and one half or four hours. The indication therefore is that a majority of the spermatozoa under natural conditions live from four to nine hours.

Table IV.

Longevity of the Sperm of *Cumingia* in Various Dilutions as Shown by the Percentage of Fertilizations that they Give with Fresh Eggs (July 25).

Age of the Suspension Tested.	1 Drop in 100 Cc. Sea Water 1/4000 to 1/6000%.	1 Drop in 50 Cc. Sea Water 1/2000 to 1/3000%.	4 Drops in 50 Cc. Sea Water 1/500 to 1/750%.	8 Drops in 50 Cc. Sea Water 1/250 to 1/500%.
2 hours	99%+	99%+	100%	100%
3½ hours	96%	95%	97%	96%
7 hours	17%	24%	99%	97%
10 hours	2%	8%	63%	72%

For this experiment the spermatozoa used were all from the same male. The eggs were from three females and were not over three hours old when used. Controls showed them to be practically 100 per cent. normal eggs.

Table V.

Longevity of the Sperm of *Cumingia* in Various Dilutions Tested on July 23.

Sperm all from one male.

Age of Sperm Suspension Used.	1 Drop in 50 Cc. Sea Water 1/2000 to 1/3000%.	2 Drops in 50 Cc. Sea Water 1/1000 to 1/1500%.	4 Drops in 50 Cc. Sea Water 1/500 to 1/750%.	6 Drops in 50 Cc. Sea Water 1/300 to 1/500%.
2¼ hours	84% cleaved	100% cleaved	100% cleaved	99%+ cleaved
4⅚ hours	35% cleaved	65% cleaved	75% cleaved	80% cleaved
7½ hours	15–20% cleaved	30% cleaved	50–60% cleaved	85–87% cleaved
9 hours	0% cleaved	0% cleaved	2% cleaved	35–40% cleaved

This table shows that sperm in 1/2000 to 1/3000 per cent. suspension died in nine hours. Other experiments have shown that from ten to forty per cent. of the spermatozoa in these most dilute suspensions often survive for 10 to 12 hours. Table IV. is more typical in this respect, but even in this case the percentage of fertilization is below the average. The point that is shown clearly is that spermatozoa die first in the most dilute suspensions and that a suspension of 1/500 to 1/750 per cent. suspension preserves the life of the sperm beyond their normal life in the open sea.

When the work of Gemmill and Lillie is taken into consideration it is apparent that Goldforb's experiments on the aging of spermatozoa do not represent normal conditions. He apparently relied upon concentrated dry sperm which are known to age much more slowly than sperm kept under natural conditions. The aging of sperm in concentrated suspensions has no particular significance. His work on the eggs is certainly not open to the same criticism but I should question his interpretation and results on the aging of spermatozoa. The work of the other two authors and this present work show that the life of spermatozoa in dilute suspensions is brief.

The statement by Lillie that the spermatozoa of *Arbacia* lose ability to fertilize eggs in a few minutes in dilute suspensions is not verified for *Cumingia* sperm, although the theory that they gradually lose something to the water which is essential to fertilization and which ultimately renders them incapable of bringing about the fertilization reaction may very well be true.

It has been my observation that spermatozoa that are able to swim actively are capable of fertilizing eggs; and that they do not lose the ability to initiate the fertilization reaction after a few minutes as reported by Lillie for *Arbacia*. Up to the present time no attempt has been made to learn whether or not the spermatozoa of *Cumingia* contain an activating substance, essential to fertilization. In many ways the spermatozoa of *Cumingia* behave as those of *Nereis* and *Arbacia* do, but if they contain a sperm receptor (Lillie, 15) preliminary experiments indicate that it is dissipated in the sea water more slowly than in the cases investigated by Lillie. Experiments on this question are in progress, as well as investigations of the cause of excessive polyspermy. These experiments are designed for comparison of the gametes of *Cumingia*, *Chætopleura* and *Hydroides* with the work of Goldforb and Gemmill on sea urchin eggs and with Lillie's work on the spermatozoa of *Nereis* and *Arbacia*.

Activation of Spermatozoa.

Spermatozoa, after having apparently lost their vitality, may be revived. When placed in a dish with eggs they become activated and swim vigorously. The activation of sperm in the

presence of eggs was observed repeatedly. The stimulus from the eggs or egg water is evident almost instantly, but not all of the spermatozoa are so activated. I interpret this to mean that most of the quiescent spermatozoa are already dead or weakened beyond the possibility of functioning.

The phenomenon of activation of apparently spent sperm by the exudations from eggs is interesting and not uncommon. The physiological value of this activation is evident although the real cause is obscure. Lillie (13) shows that the spermatozoa of *Nereis* and *Arbacia* are positively chemotropic to weak acids and to egg secretions and are apparently stimulated by them.

Concentrated Sperm Suspensions.

It was learned that spermatozoa in concentrated suspensions retain their vitality for very long periods. Under such conditions they may swim from twenty-four to thirty-six hours and give 90 to 100 per cent. fertilization and normal development. They retain sufficient life for four days to show occasional contractions visible under a compound microscope. It is evident therefore that there is some protective element in this unnatural concentration. Cohn claims that it is carbon dioxide or hydrogen ion concentration. Dry sperm of fishes has the same extended longevity although possibly from different causes, not having had the initial stimulus to swim and use up its limited store of energy.

Discussion.

Goldforb expresses the belief that deterioration of eggs begins at the time of maturation while they are still stored in the gonads. He attributes the great variability of sea urchin eggs to the differences in time that they remain in storage before spawning takes place. I find a similar variation in the vitality of eggs of *Cumingia*, but it is noteworthy that maturation does not take place in the eggs of this species until after the entrance of the spermatozoön which occurs after extrusion into the sea water. Deterioration in the gonad in this case, therefore, could not be due to maturation. The fact that eggs vary so much in their longevity leads to the belief that some deterioration takes place before extrusion and before maturation. It is hardly likely that

the great difference in vitality that experiment reveals is due entirely to natural variability. The eggs are stored in the gonads and their ducts for some time before extrusion and those that are stored longest may very well show physiological deterioration. Two cases of eggs which showed all the symptoms of aging at spawning verify Goldforb's contention on this point. At the present time I am unable to state whether the considerable variability in the longevity of *Cumingia* eggs is due principally to normal variability or is in part due to physiological deterioration while in storage. Cases of low percentage fertilization in the eggs of *Cumingia* are rare. As a rule they give 97 to 100 per cent. cleavage, although the longevity may be more variable.

Summary.

I. The average longevity of *Cumingia* eggs when kept at a temperature of 20° to 22° C. is 10 to 12 hours as judged by time of fragmentation and ability to give a high percentage of normal embryos. The average longevity at 18.5° to 20° C. is 12 to 15 hours.

II. Approximately two per cent. of any lot of eggs may be defective as shown by their early fragmentation which occurs long before the rest.

III. The outstanding fact is the wide range of variation in the vitality and longevity of *Cumingia* eggs. The eggs of a single individual vary by four hours or approximately 25 per cent., while the eggs of different females vary in their longevity from six to twenty-six hours or over 400 per cent.

IV. Eggs in rare instances show deterioration at spawning, apparently due to long-time storage in the ducts of the gonads. Most lots of eggs when freshly spawned give from 97 to 100 per cent. cleavage and normal development.

V. The longevity of the best sperm in suspensions of 1/400 to 1/500 per cent. is 10 to 12 hours as judged by functional activity and ability to give 90 to 100 per cent. fertilization and normal development. In sperm suspensions of 1/2000 to 1/3000 per cent. it is 4 to 7 hours, but from 30 to 50 per cent. fertilization may frequently be expected from suspensions 7 to 12 hours old and 1 to 5 per cent. fertilization from suspensions 12 to 20 hours old.

VI. Spermatozoa in concentrated suspensions of 1 to 3 per cent. retain their vigor for many hours. They frequently give 90 to 100 per cent. fertilization after twenty-four to thirty-six hours, and some individual spermatozoa live for four days, still showing faint contractions of the tail at intervals. This preservation of the life of the spermatozoa is attributed to the presence in the water of CO_2 produced by the activity of the spermatozoa, or to hydrogen ion concentration.

VII. Spermatozoa after becoming quiescent show activation in the presence of eggs or egg water.

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CONDITIONS DETERMINING THE ORIGIN AND BEHAVIOR OF CENTRAL BODIES IN CYTASTERS OF *ECHINARACHINIUS* EGGS.[1]

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CONTENTS.

INTRODUCTION.

Statement of the Problem Concerning the Origin of Central Bodies.

In every cell generation there are differentiated the various cell components such as chromosomes, plastids, Golgi bodies, chondriosomes, central bodies, secretory droplets and similar elements. In the case of certain ones, such as chromosomes, the synthetic processes producing new units occur only by the growth and division of preëxisting bodies of the same kind. Such a component has genetic continuity from cell to cell as an individualized body, appearing to play some rôle in its own formation. Its origin may therefore be termed autogenic or self-perpetuating. In contrast to this, the synthetic processes forming other components, such as secretory droplets, are not localized about preformed structures of the same kind. Such a component has no

[1] The experiments were performed at the Mt. Desert Island Biological Station, Maine, 1923, and at the Marine Biological Laboratory, Woods Hole, Massachusetts, 1926.

genetic continuity as an individualized body and its origin is commonly described as occurring de novo.

Central bodies[2] have special interest with reference to this problem of protoplasmic differentiation, since in some cases they arise by the growth and division of preëxistent central bodies, while in other instances they apparently are formed de novo. In some cells they persist from one cell division to another, maintaining their identity as individualized structures during the vegetative period when the mitotic mechanism, of which they appear to be the formative foci, has disappeared. At a certain time in the cell cycle they divide and the daughter cells receive central bodies that have genetic continuity with that of the mother cell. For example, in spermatogenesis, the central body of the sperm can frequently be traced as a self-perpetuating individualized structure to the central body of the primary spermatocyte.

On the other hand there is also much evidence for a de novo origin of central bodies. In higher plants where centers are absent, blepharoplasts which have the characteristics of central bodies appear de novo in the final divisions of the germ cells. In animal fertilization it has yet to be proved that the central body of the sperm aster has genetic continuity as an individualized structure with the central body of the sperm, except in a few forms such as *Cerebratulus* (Yatsu, '09), and in most cases its formation may be de novo. When eggs are artificially activated in such a way that cytasters are formed, a study of sectioned material shows that they have central bodies (Morgan, '96, '99, '00; Wilson, '01; Hindle, '10; Herlant, '19; Fry, '25*b*; Tharaldsen, '26; etc.). Since cytasters appear simultaneously in large numbers and not by division the central bodies are apparently formed de novo.[3]

[2] The term central body is used throughout this paper in its broad non-committal sense, denoting any differentiated structure present at the central region of an aster, exclusive of the inner ends of the rays (and the possible presence of chromatin). The term involves no implications as to whether or not the central body is composed of a granule, the centriole, and a surrounding zone, the centrosome, either of which may be present or absent in various organisms, and both of which may show many modifications (Wilson, '25, pp. 30 and 673).

[3] For a detailed discussion of the literature on this subject see Wilson "The Cell in Development and Inheritance," 1925, especially Chapter IX.

The idea that all central bodies are self-perpetuating structures received an impetus due to the early theory of fertilization developed by Boveri and Van Beneden. They assumed that the chief event in fertilization is the introduction by the sperm into the egg of a preformed division center and hence that the central body of the sperm aster has genetic continuity as an individualized structure with that of the sperm (Boveri, '01). So generally accepted was this assumption that when artificial parthenogenesis was discovered, where the aster appears about the egg's nucleus, it was taken for granted that the egg nucleus must therefore have such a preformed central body that is ordinarily quiescent under the normal conditions of fertilization. To explain cytasters according to this theory it was necessary either to regard them as "pseudoasters," assuming that if they do not arise about preformed centers they are not "true asters," or to assume that central bodies lie scattered through the cytoplasm as a result of hypothetical rapid divisions of the egg's hypothetical division center (Meves, '02; Wassilieff, '02; Petrunkewitsch, 04). These ideas which are without proof are only of interest to illustrate the strength of the underlying assumptions which are still generally accepted, *i.e.*, that central bodies have genetic continuity as individualized structures, and that asters ordinarily arise about such units on the theory that the central body is the persistent element of the astral mechanism acting as the formative focus.

A possible explanation of the seemingly conflicting evidence that in some cases central bodies have an autogenic origin and in others a de novo one has been proposed by Wilson. He suggests that central bodies which seem to arise de novo, may actually be self-perpetuating structures that are submicroscopic during most of their history; they grow to a visible size only at certain times when they give the impression of having a de novo origin. This suggestion is in harmony with the general assumption that central bodies are individualized structures maintaining genetic continuity and giving rise to asters. He states: ". . . the cytologist is led on to the conclusion that the ultramicroscopical dispersed particles of the hyaloplasm may be as highly diversified chemically as are the visible formed bodies;

that they may be of all orders of magnitude; and that it is they which constitute the sources, or at least the formative foci, of those larger formed bodies that we have so often, but erroneously, assumed to arise de novo. For my part, I am disposed to take a final step by accepting the probability that many of these particles (I do not say all) as if they were ultra-microscopical plastids, may have a persistent identity, perpetuating themselves by growth and multiplication without loss of their specific individual type" ('23, p. 28). "Could we accept such a view we could more readily meet some puzzling difficulties such, for example, as the apparent contradiction between the origin of a centriole de novo, and its origin by division of a preëxisting body of the same kind" ('25, p. 721).

Morgan ('96, '99, '00) has made the most careful study of the relationship between central bodies and the structure of the surrounding cytasters. As a result of his observations he raised certain questions, in sharp contrast to the generally accepted ideas, that have since been largely ignored: "Is this central deeply staining mass a distinct body, or only the innermost fused ends of the rays; or is it only a product of the reagent?" ('99, p. 477). The present study is an attempt to resolve these conflicting hypotheses concerning the origin and behavior of central bodies in cytasters.

Types of Evidence Valid for Proving a Self-perpetuating or a de novo Origin of a Cell Component.

The behavior of a cell component in hybrids yields the only conclusive evidence concerning the nature of its origin. For example, in hybrids the paternal chromosomes and their constituent genes continue to perpetuate themselves and produce their effects in the protoplasm of another species. This conclusively proves that they are autogenic, playing an important rôle in their own synthesis. It demonstrates that under normal conditions, within the protoplasm of their own species, they are not passively produced by more active elements, otherwise they would not continue to grow and divide in an alien protoplasm. There is similar evidence for the autogenic origin of certain plastids. When species with different plastids are crossed, the

paternal type continues to perpetuate itself in a cytoplasm that normally contains a different kind of plastid. The autogenic origin of other cell components has not been subjected to this conclusive proof of their capacity to perpetuate themselves by growth and division in the protoplasm of another species. Such a study of central bodies, in reciprocal crosses between *Arbacia* and *Echinarachnius*, is now in progress, a report of which will be presented in a later paper.

After reviewing the facts concerning certain plastids, Morgan ('26) states: "It may not appear far fetched to assume that there may be other bodies in the cytoplasm that grow and divide and, by extension, it might not seem too extravagant to assume that protoplasm itself (except for its secretion products) consists of units that grow and divide and are inherited." He calls attention, however, to the fact, very significant in this connection, that when two species are crossed reciprocally they produce similar hybrids. If some of the cytoplasmic components autonomously perpetuate themselves, maintaining their characteristic form, then in reciprocal hybrids the adults should show cytoplasmic differences. In one cross the cytoplasm with any self-determining units is derived from one species, and in the opposite cross it comes from the other. Since the cytoplasm of the adults is similar in both, despite its differing sources in the two crosses, this shows that its components generally are not autogenic in such a manner as to maintain their identity. It indicates that the synthetic processes are under the control of the nucleus producing similar results in both cases. It is generally to be expected, therefore, with some exceptions such as certain plastids, that cytoplasmic components do not perpetuate themselves autogenically maintaining their characteristic identity.

A component's power of growth and division, therefore, within the protoplasm of another species is primary evidence for its autogenic origin; its power of growth and division in the protoplasm of its own species constitutes a second grade of evidence indicating such an origin. Were there no genetic evidence from hybrids as to the self-perpetuating nature of chromosomes and certain plastids and were their behavior studied only in the cells in which they normally occur, the fact that they produce

new units by the growth and division of similar preëxisting ones indicates an autogenic origin. When centrioles of secondary spermatocytes are produced by the growth and division of centrioles of primary spermatocytes, this is evidence that at least they localize the synthetic processes forming new centriole substance and therefore are autogenic to that extent. In so far as there is evidence for the formation of chondriosomes, Golgi bodies and vacuoles from similar preëxisting units, to that extent is there probability of their autogenic origin.[3]

Wilson ('01) describes the division of central bodies in dividing cytasters of artificially activated eggs of *Toxopneustes*, noting the significance of this with reference to the self-perpetuating nature of such central bodies. Fry ('25*b*) studied the behavior of cytasters in nucleated and enucleated *Echinarachnius* eggs in both living and fixed material, and found division only in those instances when they secondarily established connection with chromatin. Tharaldsen ('26) found the same situation in cytasters of *Asterias*. Although a cytaster may divide, this has no significance concerning the origin of its central body unless it is conclusively proved: (1) that the central body is an individualized structure independent of the rays, and (2) that its division initiates, and is not just a result of, the division of the surrounding cytaster. Since both of these points are unproved, and since the present study brings strong evidence against the former, the facts concerning the division of central bodies in dividing cytasters are in too uncertain a state to use them as evidence of an autogenic origin.

The foregoing types of evidence concerning a component's self-perpetuating nature, based upon its power of division, whether within the protoplasm of another species or within its own, assumes that it is microscopically visible throughout its history. In those components where new units do not visibly arise by division, but apparently are formed de novo, evidence concerning their origin becomes more difficult to secure. As previously noted, they actually may be self-perpetuating bodies that are submicroscopic during part of their history, becoming visible only at certain times. "Manifestly it is quite illogical to affirm an origin de novo of any formed body because it first becomes visi-

ble at a particular enlargement, even the greatest at our present command" (Wilson, '23, p. 24). Data can be obtained, however which may strongly support either an autogenic origin or a de novo one. If a component is obviously the product of another component; if its behavior is invariably correlated with certain changes in surrounding structures, then the presumption is very strong that it is produced by them and has a de novo origin. If, on the other hand, it is obviously not the product of surrounding structures but rather is their formative and controlling factor; if it passes through a certain definite cycle which is to some degree independent of modifications in surrounding structures; such facts, depending upon the details of the evidence, may indicate its self-perpetuating nature. Data of this type were secured in the present study.

Experiments.

This investigation deals with the origin and behavior of central bodies in cytasters of artificially activated eggs of *Echinarachnius parma*, to ascertain whether they are de novo structures produced by the cytasters, or whether they are individualized structures having an identity independent of the surrounding cytaster which forms about them, and have further characteristics indicating a self-perpetuating origin. The experiments are arranged as follows: (1) keeping the environmental factors of activation constant, to observe the various effects of different fixatives upon rays and central bodies; (2) keeping the fixative constant, to observe the various effects of modifications of environmental factors such as temperature and osmotic pressure upon rays and central bodies; (3) keeping both fixative and environmental factors constant, to observe the effects of various intervals during the astral history upon rays and central bodies.

The Effects of Various Fixatives upon Rays and Central Bodies of Cytasters (Chart I.).

The eggs for this group of experiments, as well as the others to be reported later, were obtained by removing the oral surface of the animals and securing eggs from those individuals where they appeared upon the exposed ovaries. The activation techni-

EXPERIMENT NUMBER	FIXATIVE: NAME [THOSE CONTAINING ACETIC ACID ARE MARKED BY AN ASTERISK]	FIXATIVE: FORMULA	PERCENTAGES OF: NO RAYS, NO CENTRAL BODY	VAGUE RAYS, NO CENTRAL BODY	DISTINCT RAYS NOT EXTENDING TO CENTER, NO CENTRAL BODY [CENTRAL AREA STAINED SAME AS RAY AREA]
			 A	 B	 C
1	* FLEMMING-STRONG	1% Chrome acid 15 pts; 2% Osmic acid 4 pts; Acetic acid 1 pt			
2	FLEMMING-STRONG WITHOUT ACETIC	1% Chromic acid 15 pts; 2% Osmic acid 4 pts		70	
3	* FLEMMING-WEAK	Strong Flemming 1 pt; Water 1 pt		12	17
4	FLEMMING-WEAK WITHOUT ACETIC	Strong Flemming without Acetic acid 1 pt; Water 1 pt		75	
5	OSMIC ACID	.025% Osmic acid		100	
6	CORROSIVE SUBLIMATE COLD-ALKALINE	Saturated solution $HgCl_2$ - Cold - Made alkaline with NaOH		100	
7	CORROSIVE SUBLIMATE COLD-ACID	Saturated solution $HgCl_2$ - Cold - Has acid reaction	50	50	
8	CORROSIVE SUBLIMATE HOT-ACID	Saturated Solution $HgCl_2$ Heated to 90°C Has acid reaction	100		
9	* SUBLIMATE ACETIC 1%	Sat Solution $HgCl_2$ - 99 pts; Acetic acid 1 pt	15	25	2
10	* SUBLIMATE ACETIC 5%	Sat Solution $HgCl_2$ - 95 pts; Acetic acid 5 pts	38	33	
11	* SUBLIMATE ACETIC 10%	Sat Solution $HgCl_2$ 90 pts; Acetic acid 10 pts	15	50	2
12	* PICRO-ACETIC-STRONG	Sat sol Picric acid 1 pt; Water 1 pt; Acetic acid 5%		30	25
13	* PICRO-ACETIC-WEAK	Picro-Acetic-Strong 1 pt; Water 1 pt		20	15
14	* BOUIN	Sat sol Picric acid 75 pts; Formol 25 pts; Acetic acid 5 pts		10	10
15	FORMOL 5% ALKALINE	5% Formol made alkaline with $MgCO_3$		100	
16	FORMOL 5% ACID	5% Formol Has acid reaction	14	86	
17	ALCOHOL 100%	100% Alcohol	25	75	
18	ALCOHOL 35%	35% Alcohol		85	15
19	* ZENKER	Potassium dichromate 2 gm; Sodium sulphate 1 gm; Water 95 cc; Mercuric chloride 5 gm; Acetic acid 5 cc		80	
20	ZENKER WITHOUT ACETIC	Same as above without Acetic acid		100	
21	* CARNOY and LEBRUN	100% Alcohol 1 pt; Acetic acid 1 pt; Chloroform 3 pts; Mercuric chloride to saturation		5	20
22	CARNOY and LEBRUN WITHOUT ACETIC	Same as above without Acetic acid	2	45	15
23	* PETRUNKEWITSCH	Water 300 pts; 100% Alcohol 200 pts; Acetic acid 90 pts; Nitric acid 10 pts; Mercuric chloride to saturation		22	
24	PETRUNKEWITSCH WITHOUT ACETIC AND NITRIC	Same as above without Acetic and Nitric acids		94	2

CHART I. THE EFFECTS OF VARIOUS FIXATIVES

Activation: same in all experiments; a slight over exposure of the optimum butyric-hypertonic technique, cf. footnote 4, p. 372. *Fixatives:* as listed. *Times of fixation:* at frequent intervals after activation, cf. footnote 5, p. 372. *Percentages of types of cytasters:* averages of detailed counts made at the intervals at which eggs were fixed, from twenty-five minutes until one hour after activation; each count based on about 100 cytasters chosen at random from a number of eggs; examples of such data for Fixatives 3, 10 and 13, shown in Chart III, p. 380.

TYPES OF CYTASTERS AND CENTRAL BODIES

	DISTINCT RAYS NOT EXTENDING TO CENTER NO CENTRAL BODY (CENTRAL AREA STAINED LIGHTER THAN RAY AREA)  C 2	DISTINCT RAYS EXTENDING TO CENTER NO CENTRAL BODY  D	DISTINCT RAYS EXTENDING TO CENTER CENTRAL BODY VAGUELY DEMARKED GRANULAR  E 1	DISTINCT RAYS EXTENDING TO CENTER CENTRAL BODY CLEARLY DEMARKED GRANULAR  E 2	DISTINCT RAYS EXTENDING TO CENTER CENTRAL BODY CLEARLY DEMARKED CORPUSCULAR  E 3	DISTINCT RAYS EXTENDING TO CENTER CENTRAL BODY CLEARLY DEMARKED RETICULAR  E 4
*1		54		46		
2		30				
*3	12	41	11	2	3	2
4		25				
5						
6						
7						
8						
*9		30	27	1		
*10		17	12			
*11		31	1	1		
*12	20	23	2			
*13	9	20	6	29		1
*14	15	56	1	6	1	1
15						
16						
17						
18						
*19		20				
20						
*21	75					
22	2	36				
*23	55	23				
24	4					

UPON RAYS AND CENTRAL BODIES OF CYTASTERS.

Illustrations: show only the central regions, cf. Fig. 2, p. 390. *Result:* when eggs containing similar cytasters produced by similar activation are fixed by various fixatives, central bodies are present only when the fixative coagulates the cytaster in such a manner that rays are distinct and reach the center. For further details cf. p. 369. *Secondary result:* with few exceptions, only fixatives containing glacial acetic acid (indicated by an asterisk) clearly fix rays. For further details cf. p. 375.

que used was that developed by Just ('19). An egg sample of each individual was given a preliminary test of their capacity to form clear membranes by treating them with butyric acid solution (2 cc. 1/10 *N* butyric acid plus 50 cc. sea water). Only those eggs were used that gave over ninety per cent. membranes.

An artificial activation for *Echinarachnius* that is optimum for producing the maximum number of normal embryos yields only about ten per cent. eggs with cytasters (Fry, '25*a*). The activation used in the present experiments, therefore, was a slight over exposure so as to produce about twenty-five per cent. eggs with cytasters to facilitate their study.[4] The eggs show an average of seven per cent. cytolysis, although there is considerable variation as to this in the different fixatives.

Cytasters of *Echinarachnius* arise about fifteen minutes after removal from the activating solutions. None divide except occasional ones that secondarily establish contact with chromatin, and if they are numerous, cleavage does not usually occur (Fry, '25*b*). Thus the majority of cytasters formed soon after activation remain unchanged for hours except as to modifications of their detailed structure which are studied by fixing samples of eggs at various intervals.

Eggs were fixed in twenty-four fixatives being placed in each at frequent intervals after activation.[5] They were sectioned 5 μ thick and stained in Heidenhain's hæmotoxylin. Various degrees of destaining, carefully studied with water immersion

[4] Experiments 1 to 4 inc. and 7 to 14 inc., Chart I, were activated with a 35 second treatment with butyric acid solution (2 cc. 1/10 *N* butyric acid plus 50 cc. sea water), followed by a 20 minute interval in sea water, followed by an 18 minute treatment with hypertonic sea water (5 cc. 2.5 *N* sodium chloride solution plus 50 cc. sea water) at 24° C. Experiments 5 to 6 inc. and 15 to 24 inc. were similarly activated with butyric acid solution, followed by a similar interval in sea water. In treating them with hypertonic sea water (same concentration), however, the eggs were divided into four lots and exposed 15, 20, 25 and 30 minutes respectively. The experiment was so arranged that all lots came out of the hypertonic solution at the same time, and then they were mixed. The temperature was 22° C. That the two groups received an essentially similar activation is shown by the fact that samples of both contained about the same percentages of various types of development at twelve hours after activation.

[5] Experiments 1 to 4 inc. and 7 to 14 inc., Chart I., were fixed at the following intervals after activation: 15 min., 17.5, 20, 22.5, 25, 27.5, 30, 35, 40, 45, 50, 55, 1 hr., 1 hr.-15 min., 1–30, 1–45, 2 hrs., 3, 4, 5. Experiments 5 to 6 inc. and 15 to 24 inc. were fixed at the following intervals: 10 min., 15, 20, 30, 45, 1 hr., 2, 3.

lenses, show no significant differences in results. Stains other than hæmotoxylin were not used as it has been generally found that they yield similar results, and hæmotoxylin is the accepted standard.

The percentages of the various types of cytasters and their central bodies shown for each fixative, Chart I., are averages of a number of detailed counts, one made for each interval when eggs were fixed, from twenty-five minutes to one hour after activation (cf. footnote 5, p. 372). This is the period during which cytasters are at their maximum development. Each count for each interval is based upon the study (at 750 × magnification) of about 100 cytasters and their central bodies selected at random from a number of eggs. Such detailed counts for Experiments 3, 10 and 13, Chart I., are shown in Chart III., p. 380.

Since sections were cut at 5 μ, and since the majority of cytasters are 10 to 15 μ in diameter, most of them are found in two or more serial sections. In the case of each cytaster only that section was studied that includes its mid-region (Fig. 1, *b*, p. 384), ignoring the others (Fig. 1, *a* and *c*). The drawings show only the central portion of such mid-sections. The peripheral area is omitted to save space on the charts since it yields no significant information concerning rays and central bodies not shown by the central area. An entire mid-section of a cytaster is illustrated, Fig. 2, p. 390; the dotted line makes clear how small a portion of the central region is shown in the chart illustrations. Although the drawings are necessarily diagrammatic they are an accurate representation of the various classes of cytasters and their central bodies. They have the following classification (cf. Chart I.):

Rays.	*Central Body.*	*Chart Symbol.*
None	None	A
Vague	None	B
Distinct, not extending to center:		
Central area stained same as ray area	None	C1
Central area stained lighter than ray area	None	C2
Distinct, extending to center	None	D
Distinct, extending to center	Vaguely demarked—granular	E1
Distinct, extending to center	Clearly demarked—granular	E2
Distinct, extending to center	Clearly demarked—corpuscular	E3
Distinct, extending to center	Clearly demarked—reticular	E4

When cytasters that are in a similar condition due to similar activation, are fixed with a variety of fixatives, central bodies are present only when the fixative coagulates the cytaster in such a manner that the rays are distinct and extend to the center. The fact that rays are distinct and reach the center does not guarantee the presence of central bodies, since many cytasters with such rays are without them (Type D). In the majority of such cases the rays are more delicate than in those cytasters having central bodies, where are usually found rays of maximum coarseness and clarity (Types E1, E2, E3 and E4). Central bodies are never present when rays are absent (Type A), or vague (Type B), or clear without reaching the center (Types C1 and C2). Differences in the size of cytasters, which may vary from 5 to 25 μ, do not affect this relationship between the presence of central bodies and distinct central rays, nor does the location in the egg which may be central or peripheral.

Within any one egg there are found only related types such as, A and B, or B and D, etc. Unrelated types do not occur together. There is a similar occurrence of related types among the different eggs of the same slide, fixed at a given interval after activation. Since the percentages of the different types shown for each fixative of Chart I. are averages of a number of detailed counts made at various intervals after activation, the variety of types found in the general average is usually greater than those found at any one interval.

Type A, without any rays, is easily identified as a cytaster since it is a granular area that stains darker than the surrounding cytoplasm, of the same size as a typical cytaster, and is frequently associated with those having vague rays. This rayless type is often found during the late history of asters when the rays frequently fade out completely.

In this series of experiments, concerning the effects of fixatives (Chart I.), the twenty-four lots of eggs which were placed in the twenty-four killing agents, at various intervals, had received a similar activation. Hence the living eggs, prior to fixation, contained about the same array of living cytasters. Therefore the wide diversity in cytaster structure found on the slides is the result of differing coagulation products of various fixatives

which differently coagulate the same phenomena. It will be noted, for example, that Type C, with clear rays not extending to the center, is entirely absent in certain fixatives but present in others in considerable numbers. Again, some of the reagents show large percentages of Types D and E having clear central rays, whereas others show only Types A and B, which are either without rays or have only vague ones. The significance of the relation between coarse clearly-fixed central rays and the presence of central bodies will be discussed later (p. 383).

In those fixatives containing glacial acetic acid (marked on Chart I. with an asterisk) there is a total of sixty-nine per cent. cytasters with distinct rays (Types C1 to E4), and thirty-one per cent. with rays absent or vague (Types A and B). On the other hand, in those without glacial acetic acid there is a total of ten per cent. with distinct rays and ninety per cent. with no rays or vague ones. The possible significance of acetic acid with reference to the chemical composition of astral rays will be discussed in a later paper. Experiments have been carried out concerning the effects of acetic acid, related acids, and acetates, at various hydrogen ion concentrations, as well as similar studies of other fixing agents.

The Effects of Modifications of Environmental Factors upon Rays and Central Bodies of Cytasters (*Chart II.*).

In the preceding experiments the activation was constant and the fixation was varied; in the present series the fixative is constant and the environmental factors of activation are varied. Chart II. presents the various modifications of activation used, *i.e.*, treatment with both weak and strong hypertonic sea water, each for forty, sixty and eighty minutes, to study the effects of differences in osmotic pressure; treatment with butyric acid solution followed by exposure to hypertonic sea water, for ten, twenty, and thirty minutes, to study the effects of modifications of the butyric-hypertonic activation technique; modifications of temperature, at two degree intervals between 20° C. and 28° C., the range within which cytasters occur when using an optimum activation, to study temperature effects; treatment with one per cent. and five per cent. ether, each for three, fifteen and

EXPERIMENT NAME AND NUMBER		ACTIVATION DATA						PERCENTAGE OF CYT-ASTER EGGS 1 hr. AFTER ACTIVATION	PERCENTAGE OF CYTOLIZED EGGS 1 hr. AFTER ACTIVATION
		ETHER		BUTYRIC ACID	HYPERTONIC SEA WATER		TEMPERATURE C.		
		PERCENTAGE OF ETHER ADDED TO SEA WATER	LENGTH OF TREATMENT WITH ETHER AND SEA WATER MIXTURE	LENGTH OF TREATMENT WITH 2cc ¾N BUTYRIC ACID PLUS 50cc SEA WATER [FOLLOWED BY 20 min INTERVAL IN SEA WATER BEFORE HYPERTONIC TREATMENT]	NUMBER OF c c OF 2.5 N NaCl ADDED TO 50cc SEA WATER WHEN MAKING HYPERTONIC SEA WATER	LENGTH OF TREATMENT WITH HYPERTONIC SEA WATER			
MODIFICATIONS OF WEAK HYPERTONIC TREATMENT	IA	No Ether Treatment		No Butyric Treatment	5cc.	40 min.	17	5	0
	IB	"	"	"	"	60 min.	"	14	5
	IC	"	"	"	"	80 min.	"	16	10
MODIFICATIONS OF STRONG HYPERTONIC TREATMENT	IIA	No Ether Treatment		No Butyric Treatment	12cc	40 min.	17	7	22
	IIB	"	"	"	"	60 min	"	28	55
	IIC	"	"	"	"	80 min	"	35	52
MODIFICATIONS OF BUTYRIC HYPERTONIC TREATMENT	IIIA	No Ether Treatment		35 sec.	5c.c.	10 min.	17	12	0
	IIIB	"	"	"	"	20 min	"	20	0
	IIIC	"	"	"	"	30 min	"	60	0
MODIFICATIONS OF TEMPERATURE	IVA	No Ether Treatment		35 sec.	5 c.c	17 min	20	0	0
	IVB	"	"	"	"	"	22	1	0
	IVC	"	"	"	"	"	24	45	0
	IVD	"	"	"	"	"	26	.1	33
	IVE	"	"	"	"	"	28	0	85
MODIFICATIONS OF 1% ETHER TREATMENT	VA	1%	3 min.	33 sec.	5 c.c.	30 min	18	3	0
	VB	"	15 min	"	"	"	"	5	10
	VC	"	35 min.	"	"	"	"	6	14
MODIFICATIONS OF 5% ETHER TREATMENT	VIA	5%	3 min.	33 sec.	5 c.c.	30 min	18	10	30
	VIB	"	15 min	"	"	"	"	.1	40
	VIC	"	35 min.	"	"	"	"	0	83

CHART II. THE EFFECTS OF MODIFICATIONS OF ENVIRONMEN-

Activation: modified as listed. *Fixative:* all experiments fixed with saturated corrosive sublimate plus 2.5 per cent. glacial acetic acid. *Times of fixation:* as listed. *Percentages of types of cytasters:* each count for each experiment, at each of the three intervals of fixation, based on about 100 cytasters chosen at random from a number of eggs.

PERCENTAGES OF TYPES OF CYTASTERS AND CENTRAL BODIES

[FIXED WITH SUBLIMATE ACETIC 2.5%]

	½ HOUR AFTER ACTIVATION				1 HOUR AFTER ACTIVATION				2 HOURS AFTER ACTIVATION			
	NO RAYS NO CENT. BODY	VAGUE RAYS NO CENT. BODY	DISTINCT RAYS TO CENTER NO CENT. BODY	DISTINCT RAYS TO CENTER GRANULAR CENT. BODY	NO RAYS NO CENT. BODY	VAGUE RAYS NO CENT. BODY	DISTINCT RAYS TO CENTER NO CENT. BODY	DISTINCT RAYS TO CENTER GRANULAR CENT. BODY	NO RAYS NO CENT. BODY	VAGUE RAYS NO CENT. BODY	DISTINCT RAYS TO CENTER NO CENT. BODY	DISTINCT RAYS TO CENTER GRANULAR CENT. BODY
	A	B	D	E1	A	B	D	E1	A	B	D	E1
IA		90	10			10	65	25		12	72	16
IB		80	20			13	74	13	[No Experiment]			
IC		31	46	23		50	29	21	19	50	19	12
IIA	[No Experiment]					60	37	3	25	69		6
IIB	[No Experiment]					19	60	21	90	10		
IIC	[No Experiment]					7	73	20	86	14		
IIIA	No Cytasters					12	38	50		24	66	10
IIIB	No Cytasters					17	39	44	[No Experiment]			
IIIC		1	56	43		8	71	21	[No Experiment]			
IVA	No Cytasters				No Cytasters				No Cytasters			
IVB			34	66			75	25		50	50	
IVC		45	55			45	25	30		87	13	
IVD		40	60			22	78			80	20	
IVE	No Cytasters				No Cytasters				No Cytasters			
VA	17	63	15	5		20	60	20		14	26	60
VB			95	5		8	17	75		60	18	22
VC	11	33	11	45		27	21	52		24	46	30
VIA		10	15	75		60	30	10		15	45	40
VIB	20	60	20		50		20	30	29	29	40	2
VIC	No Cytasters				No Cytasters				No Cytasters			

TAL FACTORS UPON RAYS AND CENTRAL BODIES OF CYTASTERS.

Illustrations: show only the central regions, cf. Fig. 2, p. 390. *Result:* when a fixative is used capable of clearly fixing rays, central bodies are present only when the modifications of the environmental factors of activation produce well-formed cytasters with distinct rays reaching the center. For further details cf. p. 375.

thirty-five minutes, preceding a butyric-hypertonic activation, to study the effect of an anæsthetic. The chart has a column showing the percentage of eggs in each experiment containing cytasters at one hour after activation. Another column shows the percentage of cytolysis. There are wide variations in both.

The eggs of all these experiments were fixed in sublimate acetic 2.5 per cent., *i.e.*, saturated solution of corrosive sublimate 97.5 per cent. plus glacial acetic acid 2.5 per cent. They were fixed at a half hour, at one hour, and at two hours after activation. Sublimate acetic fixatives (Chart I., Fixatives 9, 10, and 11) produce four types of cytasters, with few exceptions: (1) with no rays and no central body (Type A); (2) with vague rays and no central body (Type B); (3) with distinct central rays and no central body (Type D); (4) with distinct central rays and a vaguely demarked granular central body (Type E1). The greater variety of central body structure seen in such fixatives as weak Flemming or Bouin (Chart I., Fixatives 3 and 14) are not found in fixation with sublimate acetic.

Eggs were sectioned and stained as described above (p. 372). The percentages of cytaster types shown for each experiment, at each of the three intervals after activation when eggs were fixed, are each based on a study of about 100 cytasters and their central bodies selected at random from a number of eggs. In certain experiments, however, where cytasters are found in only one per cent. of the eggs, or less (Chart II., Experiments IV. B, IV. D and VI. B), the number of cytasters studied was about twenty-five.

In this group of experiments (Chart II.) the living eggs of the various ones contained widely differing percentages of various types of living cytasters, due to the variety of astral phenomena produced by the wide modifications of activation. These differences are visible in living cytasters by the use of a high power water immersion objective. Poorly formed cytasters appear as homogeneous structureless vesicles. Although well-formed ones do not show the clean-cut thread-like rays seen in fixed material, they clearly do show a radiate configuration. These eggs with their differing cytasters were all placed in the same fixative (sublimate acetic 2.5 per cent.), in contrast to the preceding

experiments (Chart I.) where eggs with similar cytasters were placed in different fixatives. The results of both groups of experiments confirm each other. *Using a sublimate acetic 2.5 per cent. fixation, central bodies are present only when the various modifications of activation produce well-formed cytasters with distinct rays extending to the center.* This relationship holds true whether eggs having cytasters number 0.1 per cent. (Chart II., Experiments IV. D, VI. B) or 60 per cent. (III. C); whether the cytasters arise early (III. C, etc.) or late (III. A, III. B); whether there is no cytolysis (IV. A, etc.) or 33 per cent. cytolysis (IV. D, etc.); whether the cytasters are small (5 μ) and numerous, or large (25 μ) and few; whether they are located in the egg centrally or peripherally.

The meaning of the differences in percentages of types of cytasters at one half hour, one hour, and two hours after activation, will be discussed in the next section.

The Effects of Various Intervals in the History of Cytasters upon Rays and Central Bodies (Chart III.).

In the first group of experiments environmental factors of activation are constant and the fixative is the variable; in the second group the environmental factors are the variable and the fixative is constant. In the present group both fixative and environmental factors are constant and the variable is different intervals of time during the history of the cytaster.

Chart III. presents the percentages of classes of cytasters found at frequent intervals from fifteen minutes to two hours after activation, in the case of three typical fixatives where central bodies occur, *i.e.*, weak Flemming, sublimate acetic 5 per cent., and weak picro-acetic. The counts for each interval are based on the study of about 100 cytasters and their central bodies chosen at random from a number of eggs. This is the same material from which the data for Chart I. were obtained. The sectioning and staining technique were previously described (p. 372).

It will be observed that rays first appear at about fifteen minutes after activation, remaining vague for some minutes. Clear rays are not usually found until twenty minutes after

FIXATIVE	INTERVALS AFTER ACTIVATION WHEN COUNTS WERE MADE OF CYTASTERS AND CENTRAL BODIES	PERCENTAGES OF TYPES OF CYTASTERS AND CENTRAL BODIES								
		NO RAYS NO CENT. BODY	VAGUE RAYS NO CENT BDY	DISTINCT RAYS NOT EXTEND. TO CENTER NO CENT BDY CENTRAL AREA STAINED SAME AS RAY AREA	DISTINCT RAYS NOT EXTEND. TO CENTER NO CENT BDY CENTRAL AREA STAINED LIGHTER THAN RAY AREA	DISTINCT RAYS EXTENDING TO CENTER NO CENT BDY	DISTINCT RAYS EXTENDING TO CENTER CENTRAL BDY VAGUELY DEMARKED GRANULAR	DISTINCT RAYS EXTENDING TO CENTER CENTRAL BDY CLEARLY DEMARKED GRANULAR	DISTINCT RAYS EXTENDING TO CENTER CENTRAL BDY CLEARLY DEMARKED CORPUSCULAR	DISTINCT RAYS EXTENDING TO CENTER CENTRAL BDY CLEARLY DEMARKED RETICULAR
		 A	 B	 C 1	 C 2	 D	 E 1	 E 2	 E 3	 E 4
FLEMMING WEAK	15 min.		100							
	17.5 "			100						
	20 "			25		55		20		
	22.5 "			20		80				
	25 "				50	50				
	27.5 "					100				
	30 "		45	10	35	10				
	35 "			10	15	35	30		10	
	40 "			28		28	29		10	5
	45 "			8		50	17		8	17
	50 "		40	30		20		10		
	55 "		12	47		40		1		
	1 hr.		5	25		35	25	10		
	1 hr. - 15 min			50		50				
	1 " - 30 "			34	16	34	16			
	1 " - 45 "		17	16	17	50				
	2 hrs.		66			34				
SUBLIMATE ACETIC 5%	15 min	34	66							
	17.5 "	70	30							
	20 "	50	50							
	22.5 "	33				34	33			
	25 "	2	10			68	20			
	27.5 "		33			34	33			
	30 "		33			34	33			
	35 "		90				10			
	40 "	45	45			5	5			
	45 "	50	50							
	50 "	100								
	55 "	100								
	1 hr.	[No Experiment]								
	1 hr. - 15 min	60	40							
	1 " - 30 "	55	45							
	1 " - 45 "	70	30							
	2 hrs	50	50							
PICRO-ACETIC WEAK	15 min	[No Experiment]								
	17.5 "	100								
	20 "	[No Experiment]								
	22.5 "	25		30		45				
	25 "		14		44	28	14			
	27.5 "			5		95				
	30 "			20	25	5	5	45		
	35 "		25	15	13		5	40		2
	40 "		50				16	34		
	45 "							100		
	50 "		12	31		19		38		
	55 "		40	30		20	10			
	1 hr.		40	30		20	10			
	1 hr. - 15 min		40	20		20		20		
	1 " - 30 "		13	25	6	50			6	
	1 " - 45 "		23	10		35	12	12	4	4
	2 hrs		50	50						

CHART III. THE EFFECTS OF VARIOUS INTERVALS IN THE HISTORY OF CYTASTERS UPON RAYS AND CENTRAL BODIES.

Activation: same in all experiments; a slight over exposure of the optimum butyric-hypertonic technique, cf. footnote 4, p. 372; material same as that reported on Chart I, pp. 370–371. *Fixatives:* as listed. *Times of fixation:* as listed. *Percentages of types of cytasters:* each count for each interval of fixation, based on

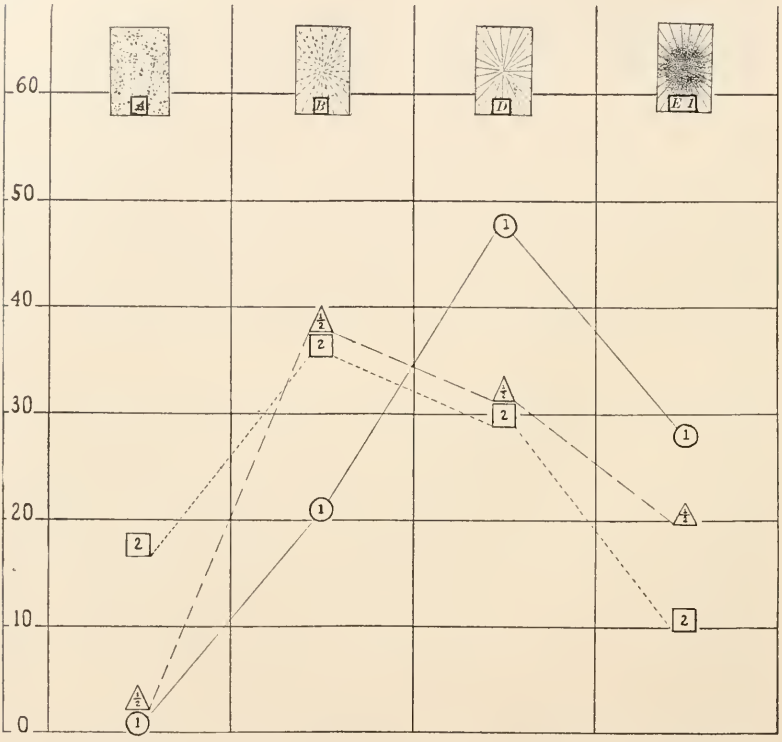
activation. Central bodies do not appear until rays have become clear, and they are never present unless the rays are distinct and reach the center. An hour or two after activation there is a tendency for the asters to become vague. When the rays become indistinct the central bodies disappear. This confirms the foregoing conclusion. *Central bodies are present only when distinct rays extend to the center of the cytaster. Cytasters pass through a formative period of vague rays when central bodies never occur; only after the rays have become clear and reach the center do central bodies appear; if the rays fade in the later history of the aster the central bodies disappear.* Thus clearly correlated with the appearance and disappearance of distinct central rays is the appearance and disappearance of central bodies. This relationship is somewhat modified by the effects of various fixatives (Chart III.) as to the percentages of types, and as to the exact time schedule, but the story is essentially the same in all.

The three fixatives reported in Chart III. are capable of showing distinctly fixed rays. The vague rays of the early and later periods of the astral history are not due to poor fixation. They are caused by the actual condition of the living rays at the time of fixation, since the same fixatives show distinct rays at the mid-period of the astral cycle. The central body phenomena shown in these three fixatives are the same as all those fixatives of Chart I. where rays are clearly fixed. Where rays are not clearly fixed there are no central bodies.

Similar results are shown in the experiments where activation is modified, Chart II. The eggs were fixed with sublimate acetic 2.5 per cent., at one half hour, at one hour, and at two hours after activation. It will be noted that in most of the experiments the highest percentage of vague (or absent) rays usually occurs at a half hour and at two hours after activation,

about 100 cytasters chosen at random from a number of eggs. *Illustrations:* show only the central regions, cf. Fig. 2, p. 390. *Result:* when an activation is used capable of producing well-formed cytasters, and a fixative is used capable of clearly fixing rays, if cytasters are studied at close intervals after activation, it is found that rays are at first vague and there are no central bodies; they appear only after rays have become clear and reach the center; they disappear when rays fade in the later history. For further details cf. p. 379.

whereas the peak of clear rays is usually at one hour. Since central bodies occur only when rays are clear and reach the center, they too show their optimum numbers at one hour, and are less numerous at one half and at two hours after activation. These relationships are shown in Graph 1. The percentages of



GRAPH 1. The effects of various intervals in the history of cytasters upon rays and central bodies.

This graph is derived from the data of Chart II., pp. 376–377. It shows the percentages of the various types of cytasters present at $\frac{1}{2}$ hr., 1 hr., and 2 hrs., after activation, when all the detailed experiments concerning the effects of modified activation are averaged together. A triangle is the symbol for $\frac{1}{2}$ hr. after activation, a circle for 1 hr., and a square for 2 hrs. Only cytaster types A, B, D and E1 occur, since the fixative used was sublimate acetic 2.5 per cent. The graph shows that asters with vague rays (or none) usually occur in maximum numbers at $\frac{1}{2}$ hr. and at 2 hrs. after activation, whereas clear-rayed ones are most numerous at 1 hr. Also, the percentages of asters with central bodies follow the same sequence, indicating that the presence of central bodies is correlated with the presence of clear rays. For further details cf. p. 381.

each of the four types of cytasters (Types A, B, D and E1), at one half hour, one hour and two hours after activation, are in each case the total of all the detailed percentages of the various experiments for that type for that interval, shown on Chart II. A few of the individual experiments do not coincide with the average behavior, but such discrepancies are accounted for by the considerable differences in astral phenomena produced by the wide variations of activation. Despite wide modifications of osmotic pressure, temperature, butyric-hypertonic treatment, and ether treatment, producing different percentages of cytaster types, different percentages of eggs containing cytasters, different percentages of cytolyzed eggs, and different sizes and numbers of cytasters, the relationship between clear central rays and the presence of central bodies holds good.

Discussion.

The Origin of Central Bodies in Cytasters of Echinarachnius parma.

The previously described experiments prove that when eggs have been similarly activated and therefore contain similar types and percentages of cytasters, if they are fixed with a variety of killing agents, central bodies are present only when the fixative coagulates the cytaster in such a manner that rays are distinctly fixed and extend to the center (Chart I.). When, on the other hand, a fixative capable of clearly fixing rays is kept constant, but the various environmental factors used in activation are modified, the same relationship holds good, and central bodies are present only when those environmental factors produce well-formed cytasters with clear central rays (Chart II.). Finally, when an optimum activation is used capable of producing well-formed cytasters, and they are fixed by a killing agent capable of fixing rays distinctly, if the cytasters are studied at close intervals after activation, it is found that they pass through an early vague-rayed condition when central bodies are never present; only after the rays become clear and reach the center do central bodies appear; and if the rays later fade, central bodies disappear. This is strong evidence that central bodies occur only if distinct rays reach the center of the cytaster (Chart III.).

Figure 1, *a*, *b*, and *c*, shows the appearance of three serial sections (each 5 μ) of a cytaster, Type E1, that was about 15 μ in diameter. Figure 1, *d*, is a schematic reconstruction of the

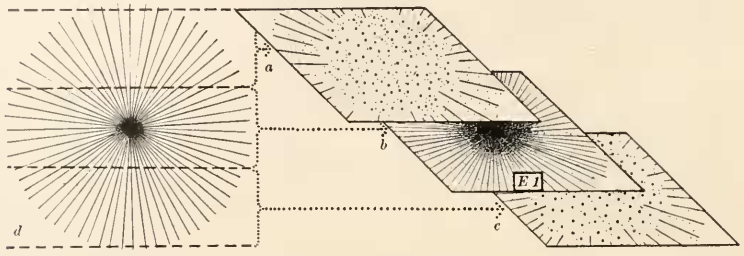


FIG. 1. The structural relations of rays and central bodies shown by a comparison of the serial sections of a cytaster with the reconstructed aster.

The serial sections (*a*, *b* and *c*), which were cut at 5 μ, are drawn from those of a cytaster having a vaguely demarked granular central body (Type E1). A schematic reconstruction of the entire aster (*d*) is shown at the left. In the top and bottom sections (*a* and *c*) rays are visible in side view in the peripheral area only, whereas in the central area they are seen in cross section as dots. In the middle section (*b*) many of the rays are visible in side view throughout their entire length, and the central portion also contains the closely crowded inner ends of the rays that extend above and below. These converge at the midpoint, and this when coagulated constitutes a central configuration, *i. e.*, the central body.

entire aster. It is obvious that the top and bottom sections (Fig. 1, *a* and *c*) present a side view of rays in only the peripheral area, and that in their central regions the rays are seen in cross section as scattered dots. It is equally apparent that the middle section (Fig. 1, *b*) presents a side view of many of the rays throughout their entire length from the edge of the cytaster to its center, and that the center also contains the inner ends of those rays extending above and below which appear in cross section as closely crowded dots.

The following hypothesis is therefore proposed as the probable explanation of the so-called central bodies seen at the center of fixed cytasters. The rays converge at the mid-point of a cytaster. When they are coagulated by fixation their closely crowded inner ends are sufficient to explain a dark configuration at the center. Since cytasters differ as to number and coarseness of rays, there is a variety of coagulation products at the mid-

points; they may be granular, corpuscular or reticular. *This study has conclusively proved that central bodies are de novo structures produced by the cytaster only after it has formed clear rays reaching the center. There is strong evidence for the further conclusion that these structures are produced only by coagulated cytasters and that they are nothing but the fixed closely crowded inner ends of well formed rays, and that they have no existence as individualized structures in the living cytaster.*

This explanation of central bodies in cytasters is supported by the fact that the type of cytaster with clear rays reaching to the center which nevertheless is without a central body (Type D), usually has rays that are more delicate than those of cytasters with central bodies (Types E1, E2, E3 and E4). Also if rays extend to the center but are vague, there are no central bodies (Type B). Therefore, only coarse well-formed rays form central bodies.

That central bodies are nothing but the coagulated bases of rays finds further support from the fact that not only must the rays be well formed, but they must also extend to the center. That type of cytaster with coarse distinct rays that do not reach the center (Type C) is of special significance in this connection. Were it not for this type it might be legitimately concluded that central bodies are individualized structures with a chemical nature similar to that of the rays; hence when rays are poorly and vaguely fixed, central bodies fail to be coagulated and appear to be absent; only when rays are well fixed are central bodies shown. The existence, therefore, of large numbers of cytasters with well-fixed distinct rays in the peripheral region that do not reach the center, disproves this possible interpretation since such cytasters never contain central bodies. If the assumption is correct that fixatives which fix central bodies are ones that fix rays, then central bodies should be present in this type of cytaster with well-fixed peripheral rays.

Almost conclusive evidence supporting the hypothesis that central bodies of cytasters are nothing but the coagulated inner ends of well-formed rays is found in the history of cytasters (Chart III.), where the appearance and disappearance of clear central rays is invariably accompanied by the appearance and disappearance of central bodies.

If, in the light of the above evidence, it is still claimed that central bodies of cytasters have an autonomous independent existence beyond the limits of microscopic vision, becoming visible only at certain times, then it must be assumed further that they always attain visibility simultaneously with the formation of distinct rays reaching the center, and again become submicroscopic whenever the rays fade; that they never attain visibility if clear rays are formed that fail to extend to the center or if vague rays arise that do reach the center. Were the central bodies of cytasters independent units having existence apart from the rays, their presence would not be so invariably dependent upon clear rays that reach the center. The wide fluctuations in structure and behavior of both central bodies and the surrounding rays caused by various fixatives, by modifications of environmental factors, and by different periods in the astral history, would not show the invariable correlation that they do. There would be at least some faint indications of central body behavior independent of clear rays reaching the center.

Although Morgan ('96, '99, '00) and Wilson ('01) did not reach such a conclusion, their numerous detailed observations of the behavior of central bodies and cytasters in *Sphærechinus*, *Arbacia* and *Toxopneustes* are the same as those of the present study. They note that cytasters are formed by the accumulation of a substance that only later assumes a radiate structure (Morgan, '96, p. 356; '99, p. 465, etc.; Wilson, '01, p. 558); that central bodies are not present at first and make their appearance only after the aster has passed through its early history (Morgan, '99, pp. 470 and 477; Wilson, '01, p. 558); that they are most apt to be present in well-formed cytasters (Morgan, '99, p. 477) whereas poorly developed ones do not contain central bodies (Wilson, '01, p. 560); that they are not present in all cytasters and show a considerable variety of structure (Morgan, '96, '99; Wilson, '01; numerous references); and that cytasters frequently fade out in their later history (Morgan, '99, p. 464; Wilson, '01, p. 554). As a result of these facts Morgan ('99, p. 477) tentatively proposed as a possibility, the conclusion established by the present study: "Whether the

centers first form and the fibers (rays) arrange themselves around them, or whether the centers are the result of the focusing of the first formed rays at a central point is difficult to determine, for both centers and rays appear at about the same time. All things considered I am inclined to adopt the latter alternative."

The plates of Morgan ('96, '99) and Wilson ('01), as well as those of other investigators illustrating echinoderm cytasters (Herlant, '19; Tharaldsen, '26, etc.), show a diverse array of cytasters and central bodies, most of which are similar to those found in the present study of *Echinarachnius*. *Toxopneustes* (Wilson, '01), however, shows certain differences. Professor Wilson kindly permitted an examination of some of his slides, and it is clear that *Toxopneustes* cytasters contain a high percentage of a central body type somewhat like E2 of the present study, but it is more compact and very much smaller. In some cases it is granular; in others it is homogeneous and is identical in appearance with a typical period-like centriole (Wilson, '01, plates XV. and XVI.). Such differences, however, due to different species, are probably of no more significance that those caused by different fixatives or differing environmental conditions. Any variations in central body structure of cytasters, no matter what the cause, are meaningless, provided the conclusion of the present study is true, that such central bodies are nothing but various coagulation products of the inner ends of well-formed central rays, having no existence as individualized structures in the living cytaster. All previous investigators of echinoderm cytasters, from Morgan ('96) and Wilson ('01) to Fry ('25*b*) and Tharaldsen ('26), have assumed that the central bodies seen in fixed cytasters are structures having an actual existence in the living egg. The present study of *Echinarachnius* proves that in this species, at least, they are but the coagulated focal point of rays. It is probable that this is true of central bodies in cytasters generally, but proof of this awaits the application of a similar method of study to cytasters of other species.

The Method of Study and its Significance for Cytological Research.

If a cell component is studied in a fixed condition it is of course necessary to check such observations with a study of living

material if this is possible. The study of living cells stained with vital dyes may yield information concerning the structure of a component when alive, to use as a basis of comparison for its appearance when coagulated. It is further necessary, if possible, to secure data concerning its chemical composition. Some components such as central bodies, are so small, however, that they cannot be seen in the living cell. Observations of such structures can be made only after the material has been coagulated and sectioned. When such is the case the greatest caution must be used in interpreting the results, keeping in mind the fact that a component when coagulated may present a very different appearance from the same component when alive.

The present study shows that various fixatives cause such a diversity of structure and behavior of central bodies, that to draw conclusions from observations made by the use of one or two killing agents might easily lead to erroneous results. A description of their structure based on fixation with Bouin's fluid would not harmonize with the results produced by sublimate acetic, and would be equally different from conclusions based on phenomena seen in material fixed with strong Flemming's fluid (Chart I.). Not only are the types and percentages of cytasters and their central bodies modified by the fixative, but the time schedule of events during the astral history is also changed. Clear rays and central bodies disappear at forty minutes after activation when eggs are fixed with sublimate acetic 5 per cent., whereas in weak Flemming's solution and in weak picro-acetic they are present until about an hour and a half after activation (Chart III.). Since the eggs were similarly activated, these time differences in the astral history are caused by the fixatives. The varying effects of different killing agents are also shown by the different percentages of cytolysis they cause, the data for which are not shown on Chart I. In the different sets, cytolysis varies from none to about fifteen per cent. at forty-five minutes after activation, and shows a much wider variation at later periods, depending upon the fixative used. Of all the factors of the environment capable of modifying the structure of a component, certainly no one of them can effect more radical changes than the killing agent. Hence if a component is studied only

in a coagulated condition it is essential to know the modifications produced not only by one or two fixatives, but by a variety of typical ones. Only when these are known is there any hope of arriving at a conception of what is a "normal" condition, and even then it must be accepted with the greatest caution.

Not only is it important to know the effects of different fixatives, but it is equally important that in the case of each fixative, accurate counts be made of all the variations of a component produced, omitting none. All the coagulation products must be considered in arriving at a result and the percentages of each should be known. Had the method of study in the present investigation been one of random search, selecting certain types as "normal" and dismissing others as "artifacts," and had all the variations in cytaster and central body structure not been studied quantitatively, it is doubtful if the real relationship between clear central rays and the presence of the central body would have been apparent. Had that type with clear peripheral rays not reaching the center and having no central body (Type C), and the type with clear central rays without a central body (Type D), as well as vague rayed asters (Type B), been passed by as due to poor fixation, and had attention been centered only on those with central body structure, the real relationship between clear central rays and central bodies would probably not have been established. It cannot be said that any of the types are more or less important than the others, or that any are "normal" or "abnormal," in arriving at the conclusion.

That cytaster with clear peripheral rays not reaching the center, in which the central area is homogeneous and stained the same as the ray area (Type C1) occasionally contains a minute deeply staining granule at its mid-point (Fig. 2). This satisfies all the requirements of one of the a priori conceptions of a "normal" central body structure, *i.e.*, one having a period-like centriole, surrounded by a homogeneous zone, the centrosome, about which is the ray area. In fact, the present investigation began on this assumption, resulting in confusion due to the inability to explain the other types which were abundant in well-formed asters. That the central granule of this cytaster

(Fig. 2) is not a centriole, and that this aster is without significance for an understanding of central bodies in cytasters is shown by the following facts. (1) These granules are found only

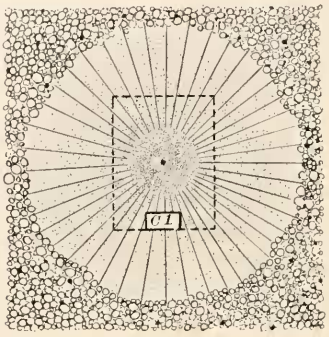


FIG. 2. Cytaster with distinct rays in the peripheral region only (type C1) containing a cytoplasmic granule at its midpoint.

This cytaster strikingly simulates a "typical" central body structure, one having a period-like centriole, surrounded by a structureless zone called the centrosome, about which is the ray area. Such a cytaster is without significance, however, concerning central body phenomena in cytasters. It occurs in but 0.5 per cent. of all cytasters. The granule is identical in appearance with those scattered through the cytoplasm. Two or three or more granules occur in such asters as frequently as does a single one.

The area within the dotted lines indicates that small portion of the midsections of cytasters shown in the chart illustrations, cf. p. 373.

in those cytasters where the rays do not reach the center (Type C1) and only in very few of them. In fact this cytaster with a centriole-like granule occurs in but 0.5 per cent. of all cytasters. Its relative scarcity was not appreciated until accurate counts were made of all the types. Until such a procedure is followed an observer can center attention upon a certain condition as typical and largely pass by other forms, and just because attention is centered on it, it may seem to be far more abundant than it actually is. Whether it occurs with a rarity that is significant can only be revealed by comparative counts. (2) The presence of a centriole-like granule in any one cytaster has no correlation with its presence or absence in other cytasters of the same egg. It is not a condition of central body structure difficult to fix, otherwise it would appear more frequently in the wide variety of fixatives used, and when present in one cytaster of an egg it

would tend to be present also in others. (3) It is important to note that such granules occur in pairs and groups of three or more as frequently as they occur singly. Wilson ('01, p. 558) observed irregular numbers of centriole-like granules, especially in the early history of cytasters. He also notes (p. 560) that in well-formed ones they may be double. (4) Most important of all is the fact that they are identical in size and appearance with the darkly-stained granules that are abundant in the cytoplasm generally. Wilson ('01, p. 557) carefully notes that granules, sometimes occurring at the centers of cytasters, are similar to those scattered through the cytoplasm. In view of the preceding points, it is clear that in *Echinarachnius* cytasters such granules that simulate centrioles are actually cytoplasmic granules that happened to be at the center of an area where an aster formed; they remain there only if the differentiation of rays does not extend to the center (Type C); they are not found at the mid-point of cytasters with clear rays reaching the center (Type D) but these contain only the much larger central bodies of a very different character previously described and illustrated (Types E1 to E4). These centriole-like granules are crowded out of cytasters if the differentiation of clear rays extends to the center, by the same forces that eliminate all relatively large particles from between well-differentiated rays in asters generally.

Morgan ('99, p. 475) notes the effects of the several fixatives he used upon the variety of central body structure seen on the slides. As a result of this he raised doubts that have been confirmed by the present work: "How large a part does the reagent play? How much is artificial and how much actually represents what is actually present in the living egg? As to nucleus and chromosomes, all series show the same results. As to cytasters, and especially as to central bodies, the door is open to skepticism." *The present investigation is a further warning of the necessity of distrusting the coagulation products of certain cellular components such as central bodies, chondriosomes, Golgi bodies, etc.; of realizing that the phenomena seen on slides may be radically different from the living condition. On the other hand, it shows with equal clearness that results may be dependable, if the various typical coagulation products are known, if all the*

types produced are studied quantitatively, and if they are all taken into consideration in arriving at a conclusion.

No profitable comparisons can be made between central bodies of cytasters and those of nuclear asters in *Echinarachnius* until the latter have been studied in a similar manner. The central bodies of the nuclear asters on the slides of the present experiments with artificially activated eggs, as well as those of cytasters that secondarily established connection with chromatin, have not been studied in detail, awaiting a controlled and quantitative study of central bodies in the nuclear asters of fertilized eggs. This is now in progress; a report of it will be presented in another paper together with a study of central bodies in fertilized eggs of *Arbacia* and *Asterias*.

Central bodies of nuclear asters in general show a great diversity of structure and behavior. In some forms they are described as autogenic, in others they appear to arise de novo; in some they seem to give rise to the aster, in others they appear only after the aster has been formed; in some they persist throughout the cell history, in others they disappear at certain phases of the cell cycle. In the light of the present study, this diversity of behavior, not to mention the variety of structure and function in the non-mitotic rôles of central bodies, makes it the more necessary to approach their future study in a controlled and quantitative manner. It is possible that if the present method is applied to the study of central bodies generally, that certain of the previous observations may be found to be based upon a single type arbitrarily selected as "normal" when using but one fixative, without knowing its quantitative relationships with respect to other types present on the same slides, and without knowing the differences caused by other fixatives. Whether or not such is the case awaits further study.

Résumé.

1. Some cellular components, such as chromosomes, arise by the growth and division of preëxisting bodies of the same kind, maintaining genetic continuity as individualized structures from cell to cell. They, therefore, have a self-perpetuating origin. In contrast to this, other components, such as secretory droplets,

have a de novo origin, since the synthetic processes forming new units occur without reference to preformed bodies of the same kind.

2. Central bodies (a term used in its broad sense without reference to various detailed modifications) are of special interest to this problem of protoplasmic differentiation, since there is evidence that in some cases their origin is de novo, while in others it is self-perpetuating.

3. Are central bodies of cytasters in artificially activated eggs of *Echinarachnius parma*, de novo structures produced by the aster, or are they self-perpetuating bodies, that are usually submicroscopic, becoming visible only at certain times and giving rise to the aster?

4. One series of experiments proves that when eggs have been similarly activated and therefore contain similar types and percentages of cytasters, if they are fixed with a variety (twenty-four) of killing agents, central bodies are present only when the fixative coagulates the cytaster in such a manner that rays are distinctly fixed and extend to the center.

5. A second series confirms the same result. When a single fixative capable of clearly fixing rays is used, but there are modifications of the various environmental factors of activation, such as temperature, osmotic pressure, etc., central bodies are present only when those environmental factors produce well-formed cytasters with clear central rays.

6. A third series also supports the preceding conclusion. When an optimum activation is used capable of producing well-formed cytasters, and they are fixed by a killing agent capable of showing distinct rays, if cytasters are studied at close intervals after activation, it is found that they pass through an early vague-rayed condition when central bodies are never present; only after the rays become clear and central do central bodies appear; and if the rays later fade, which frequently occurs, the central bodies disappear.

7. The cytaster, therefore, produces the central body only after it has formed clear rays reaching the center, no matter what are the modifications caused by fixatives, or environmental factors, or intervals of time in the astral history. Thus the

central body has a de novo origin. It is probable, moreover, that only a coagulated aster produces a central body and that it has no existence as an individualized body in the living cytaster. The mid-point of the aster contains the crowded converging inner ends of the rays. These when coagulated form the so-called central bodies seen at the centers of cytasters in fixed material. This hypothesis is supported by the following facts: central bodies are most abundant when rays are coarse, and are usually absent when rays are delicate, and always absent when rays are vague; there are many cytasters with coarse rays in the peripheral portion which do not reach the center, and these never contain central bodies; clearly correlated with the appearance and disappearance of clear central rays during the history of cytasters, is the appearance and disappearance of central bodies.

8. The investigation is another illustration of the need of a controlled and quantitative method in cytological study. When a cellular component is too small to be studied in the living condition, and must be studied only after coagulation, it is necessary not only to control the environmental factors, and to study it at frequent intervals during periods of significant change, but it is also necessary to know its various coagulation products formed by different typical fixatives. It is equally essential to know the exact percentages of all these variations, without exception, that they may all be taken into consideration quantitatively when arriving at a conclusion. To arbitrarily select a certain type as "normal" because it happens to coincide with a certain a priori idea, and to disregard the other types as "artifacts" produces uncertain results.

9. No profitable comparison of central body phenomena in cytasters of *Echinarachnius* can be made with those of its nuclear asters until the latter have been studied in a similar controlled and quantitative manner, a report of which will be presented in another paper.

10. Glacial acetic acid was present in some of the fixatives used and absent in others. Of all types of cytasters having clear rays (whether with or without central bodies), eighty-five per cent. are found in the presence of acetic acid. The possible

significance of acetic acid with reference to the chemical composition of astral rays is under further investigation.

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ACTION OF SALTS ON *FUNDULUS* EGG.

I. The Action of Na, K, and Ca Chlorides upon the Egg of *Fundulus*.

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Interest in the fundamental action of chemicals upon the egg, egg membrane, embryo, and larva of the marine fish *Fundulus* has centered largely around the work of Loeb and his associates (1–17). The results of this work seem to show that marked differences exist in reactions of the eggs and hatched embryos or larvæ to different salts. Marked degrees of salt antagonism are also pointed out. Inasmuch as the conclusions drawn from this work are based almost entirely upon the egg and hatched embryo or larva, it was thought highly desirable to recheck the observations, using eggs and embryos dissected out of the eggs, as well as hatched embryos or larvæ. By means of an operative technic devised by Doctor J. Nicholas [1] of Yale University, the details of which will be published elsewhere, it has been found possible to remove the outer egg membrane from the embryo and yolk sac without, in any apparent way, interfering with the further development of the embryo. As a matter of fact, embryos removed from the egg develop in sea water in as normal a way as those enclosed in the egg. It has thus been possible to compare the action of various salts directly upon the egg, the embryo dissected free from the egg membrane, and the larva hatched from the egg.

That Loeb conceived of a similarity in the action of salts upon the hatched embryo or larva and the embryo contained in the egg can easily be gathered from the following passages. In 1911 he says (6): ". . . if the fish is *out of the shell* the addition

[1] The writer is deeply indebted for the assistance rendered by Doctor J. Nicholas who was kind enough to carry out the operative technic involved in removing the egg membrane.

of $CaCl_2$ alone is no longer sufficient and the addition of KCl also becomes necessary." Other remarks in the same article seem also to indicate that the marked differences in the reactions to salts between the larva and embryo were not appreciated. In 1916 he says (8): "This prolongation of life through the addition of Ca is due not to an action upon the protoplasm but to a prevention of the diffusion of the NaCl into the egg, since if we take the embryo out of the egg (or use the newly hatched embryo) it is killed in 50 cc. 3 *M* NaCl + 1 cc. 10/8 *M* $CaCl_2$ inside of a few minutes." In his last paper on *Fundulus* (17) he says: "In 1905 the writer suggested as an explanation that a pure NaCl solution, if its concentration exceeded a certain limit, made the membrane of the egg more permeable, so that NaCl could diffuse into the egg, killing the embryo, while this increase in permeability was prevented by the presence of a low concentration of Ca." These and similar passages throughout his works seem to the writer to show that the marked differences in the reactions to salts between the embryo dissected out or freed from the egg membrane and the larva were not fully taken into consideration.

The present paper embodies results obtained from investigations carried out during the summer of 1927 at the Biological Laboratory, Cold Spring Harbor, on the effects of Na, K, and Ca chlorides on the eggs, embryos freed from the egg membrane, and larvæ of *Fundulus heteroclitus*.

Methods.

The eggs were stripped directly from the female fish into finger bowls containing sea water and then fertilized. They were kept at room temperature (20°–25° C.) as well as at lower temperatures (15°–18° C.). Those at the lower temperatures naturally took longer to reach a given stage in development than those kept continuously at the higher temperatures. The eggs were usually washed for varying periods in distilled water before use in order to free them of adhering salts as suggested by Loeb (13–17). This procedure, however, seems to the writer necessary to but a limited degree, since similar reactions are given by eggs washed for varying periods except when the time factor is

greatly lengthened, *i.e.*, to several days as was the case in many of Loeb's experiments (13–17).

The egg membrane was cut mid-laterally by means of fine pointed iridectomy scissors and the contained embryo gently rolled out of the shell by means of a fine probe. Eggs in as nearly as possible the same stages of development were used together with embryos dissected from similar eggs. It is of considerable importance that eggs and embryos be in the same stages of development, since marked age differences in reactions to some salts seem to exist. The length of exposure of the egg to water is also an important factor in changing the general consistency of the egg membrane. Both eggs and embryos were placed in covered Syracuse watch-glasses in 10 cc. of the solution and were constantly observed under a compound microscope during the course of the experiments. Five to ten organisms were used in a single watch-glass. It is of much importance to carry through experiments until the eggs hatch or the organisms die since in many cases the eggs live in certain solutions but upon further development and hatching the embryos quickly succumb. The embryos and eggs used ranged in developmental stages from those in which heart action was just beginning to those with fully developed circulation and ready to hatch. Newly hatched larvæ were used for comparison with the dissected-out embryos and eggs. The end-point observed and recorded in all the experiments herein reported was the time of cessation of the heart beat. Recovery of the heart beat in sea water was also noted but will be dealt with in a subsequent communication. The salts used in these experiments were c.p. NaCl, $CaCl_2$, and KCl made up in distilled water. Only the effects of normal solutions of these salts will be presented at this time since the results at this concentration are typical.

Results of Experiments.

Since in general the results of all experiments are qualitatively alike only typical experiments will be described.

N KCl.—KCl, as repeatedly pointed out by Loeb and his coworkers (1–17), acts with considerable rapidity on the egg, dissected-out embryo, and larva. In all, the heart quickly

TABLE I.

Salt.	Egg.	Embryo Freed from Membrane.	Larva.	Remarks.
N KCl.....	+ (75 min.)	++ (15 min.)	+++ (4–6 min.)	Penetrates membrane rather slowly
N $CaCl_2$.....	+ (98 min.)	+ (51 min.)	++ (30 min.)	Penetrates membrane rather slowly
N NaCl.....	young + (24 hrs.) older ○	○	+++ (10 min.)	Penetrates membrane rather slowly
Equal parts:				
N NaCl + *N* $CaCl_2$..	○	+ (12–24 hrs.)	++ (22 min.)	No penetration
N KCl + *N* $CaCl_2$...	++ (30 min.)	++ (20–25 min.)	++ (20 sec.)	Aids penetration
N NaCl + *N* KCl...	+ (73 min.)	++ (38 min.)	+++ (15 min.)	Penetrates membrane

Explanation.—Shows general conception as to toxic action of salts upon eggs, embryos and larvæ. + = relatively slow toxic action; + + = a faster toxic action; +++ = a marked toxic action; ○ = non-toxic. Figures show average time for cessation of heart beat.

stops beating, the relative order of resistance being, larva < embryo < egg. The egg is about 4 times as resistant as the dissected-out embryo, and about 20 times as resistant as the larva. In the action of KCl upon the embryo within the egg, about three fourths of the time necessary to cause cessation of the heart beat is spent in the passing through the egg membrane (Table I.). Eggs with embryos in which the heart is just beginning to beat appear less resistant then older eggs.

N $CaCl_2$.—$CaCl_2$ is also extremely toxic for eggs, embryos, and larvæ, the relative order of resistance being larva < embryo < egg. The egg is about one half as resistant as the embryo, and about 3 times as resistant as the larva. Relatively less time is spent by the Ca in going through the egg membrane than by K, since the embryo outside of the egg is killed in less than one half of the total time required for cessation of the heart beat of the embryo within the egg membrane. No marked age differences in resistance to $CaCl_2$ seem to exist, since the organisms are killed at all embryonic stages in almost the same relative time.

N NaCl.—The action of NaCl is by far the most interesting of those studied, since the results obtained are quite different from those reported by Loeb (17). The relative resistance to NaCl is, larva < embryo < egg. This series, however, must be modified because with NaCl the question of age enters in a most amazing way. Freshly fertilized eggs, as pointed out by Loeb (2), are killed in solutions of NaCl. As the egg grows older its resistance to NaCl increases up to the time of hatching. Eggs in which the embryonic heart has just begun to beat are susceptible to NaCl, while the same embryo removed from the egg will usually live for days in the same solution. As a matter of fact, they live almost as long as it takes for the normal embryo in sea water to reach the time of hatching. The freshly hatched larvæ, however, are killed very quckly when put into the same NaCl solution (Table I.). A marked change in the resistance of the animal to NaCl thus takes place when the embryo is ready to emerge or emerges from the egg.

Equal parts N KCl + N $CaCl_2$.—In such a mixture the toxicity for the egg, embryo, and larva is about the same (Table I.).

The time taken to cause cessation of the heart beat, in the mixture however, is quite different from that required in the case of solutions of the individual salts, as is shown in Table I.

Equal parts N NaCl + N $CaCl_2$.—In this mixture the larva is killed in approximately 20 to 25 minutes. The embryo dissected from the egg membrane can survive from 6 to 24 hours while the eggs are not killed. As a matter of fact, eggs will hatch in the solution, and the hatched larva is quickly killed, showing quite conclusively that while in the egg the embryo is protected; once out, it quickly succumbs. The embryo dissected from the egg membrane is always found to be much more resistant than the larva (Table I.).

Equal parts N NaCl + N KCl.—Such a mixture is quite toxic for the egg, embryo, and larva, the relative resistance being: larva < embryo < egg. The egg is approximately 2 times as resistant as the embryo and about 5 times as resistant as the larva (Table I.).

Discussion.

The above results seem to the author to be of interest inasmuch as they definitely point out and show that any assumption as to the interior condition of the egg cannot be relied upon until satisfactorily tested and proven. Loeb (1–17), in most of his work on *Fundulus*, seems to have assumed that the resistance of the hatched embryo or larva was comparable to that of the embryo within the egg membrane. In the case of NaCl in particular this view is shown to be erroneous, and it is quite possible that further investigation will yield equally interesting results. That the egg membrane changes in consistency with age is quite apparent in the ease with which the membrane can be cut. Young fertilized eggs have membranes quite tough and turgid; eggs exposed to distilled water and at low temperatures for long periods of time have much softer and more pliable membranes. Associated with these structural changes are doubtless the marked physiological ones observed. Further proof of marked physiological changes in the egg membrane have come out of unpublished investigations recently conducted by Miss E. Yagle of this Laboratory, on the exosmosis of H_2O from the *Fundulus* egg of different ages. Loeb (6), also points out in

several instances that such changes occur—*e.g.*, ". . . since the *newly fertilized* egg is killed more rapidly by a *m*/2 solution of NaCl than it is killed by the same solution one or two days after fertilization." Young eggs, therefore, are very susceptible to NaCl while older ones are quite resistant. Young embryos, on the other hand, are much less susceptible than are very old ones. The hatched embryo or larva, however, is extremely susceptible to NaCl. The question as to the fundamental action of NaCl upon the younger eggs with hearts just beginning to beat apparently is not one of a purely chemical nature but rather of an action on the membrane in which the embryo is doubtless killed by some secondary effects, possibly osmotic. In eggs thus killed the contained embryo always appears much shrunken in size.

By means of the dissection technic in liberating the embryos from the egg it is possible to study quantitatively the relative effects of the salt upon the membrane and contained embryo and also upon the embryo free from the egg membrane. KCl, for example, seems to spend about 75 per cent. of its total time effect upon the egg membrane and approximately 15–50 per cent. on the embryo. $CaCl_2$ on the other hand, spends about 60 per cent. on the membrane and 30–40 per cent. on the embryo. NaCl must exert most of its effect on the membrane in those cases in which it is toxic since for embryos removed from the egg it is relatively non-toxic.

The site of action of the three salts Na, K, and Ca chlorides has always been of considerable physiological interest. Loeb (17), in the case of *Fundulus*, seems to have attributed the fundamental action of these salts to the membrane—the process being purely a diffusion phenomenon. In young eggs NaCl perhaps does not penetrate the membrane while in older eggs the membrane seems freely permeable, since the contained embryo is not killed in the same solutions when removed from the egg. KCl and CaCl seem to penetrate the egg membrane, the Ca entering in a relatively slightly shorter time than the K. The Na + Ca antagonism as suggested by Loeb (2) must be a membrane phenomenon since the embryo removed from the egg or the hatched larva is quickly killed in such a mixture. Combi-

nations of Na + K, however, act more like KCl alone and little if any antagonism seems to exist (Loeb, 1, 2). The combination K + Ca, on the other hand, seems to exert additive effects and to kill eggs and embryos in almost the same time.

Several additional facts noted in these experiments are of especial interest since they seem intimately concerned in any fundamental explanation of the salt effect upon the egg and embryo. The space between the egg membrane and embryo is at first very small, due to the large size of the yolk sac. As the embryo develops rather a large amount of fluid accumulates between the egg membrane and embryo. This increase in fluid perhaps has much to do in modifying the rates at which the embryo is killed while in the egg. Around the yolk sac and embryo there is also a delicate vitelline membrane, the properties of which seem of much importance in respect to the resistance of the embryo to various salts. If with a very fine pointed needle a minute hole is made in this membrane immediately ventral to the eye and the embryo, still normal, transferred to a solution of NaCl or left in a solution of NaCl which is not ordinarily toxic, it is quickly killed. It seems that this membrane is an important factor in determining the resistance of the embryo to NaCl. Further investigations are to be carried out on these points.

Summary.

(1) By means of an operative technic it is possible to remove the egg membrane from the egg of *Fundulus heteroclitus* and to compare experimentally the action of salts on the egg, the embryo freed from the membrane and the newly hatched larva.

(2) The effects of normal solutions of K, Na, and Ca chlorides upon the above are reported.

(3) The embryo, freed from the egg membrane, is quite resistant to NaCl solutions while the hatched larva is quickly killed in the same solution.

(4) The resistance of the eggs to NaCl increases with age.

(5) K and Ca chlorides kill the dissected-out embryo much more quickly than the egg, while the recently hatched larva is much more sensitive to the two salts than is the embryo.

(6) Combinations of these salts show antagonistic action.

Na + Ca mixtures are not toxic for eggs but are markedly so for the embryo freed from the egg membrane and for the newly hatched larva.

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ON THE ABILITY OF CERTAIN MARINE INVERTEBRATES TO LIVE IN DILUTED SEA WATER.

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There are many reasons for believing that animal life originated in the ocean and has gradually spread through the ages into freshwater and land habitats (15, 21). In the past annelid worms invaded the soil, probably by a rather indirect route which led them first into freshwater and gradually out into the land (23). At the present time many animals in various parts of the earth show varying degrees of ability to live in diluted sea water (1, 3, 10, 11, 12, 21). Marine invertebrates which have been studied have an osmotic pressure in their blood which is approximately equal to that in sea water, but the mineral salts are somewhat less and the pressure is maintained by other substances, largely organic, which are present (1, 8, 9). The skins of various animals differ greatly in ability to control the exchange of chemicals between the body fluids and the surrounding medium. Adolph (1) studied the exchanges of substances through the skins of annelids and found that there was little resistance to them.

The writer felt that it would be of interest to determine the ability of representative marine annelids to live in diluted sea water, and during August 1927 made some observations in the Marine Biological Laboratory at Woods Hole. Thanks are due to Drs. J. A. Dawson and R. Bennitt who made suggestions and helped in the identification of several species. Mr. A. M. Hilton and his staff of collectors also made special efforts to secure materials.

Animals were brought in fresh from the field and placed as soon as possible in clean glass finger bowls containing 250 cc. of water. Tubicolous worms were removed from their tubes, except in the case of Hydroides, which was studied both in and out of tubes, and of Cystenides, which was left in its own tubes.

The water in all bowls was changed each morning, and oftener when it showed indications of becoming stagnant or when a worm bled. Dilutions were made with fresh water from the taps in the Marine Biological Laboratory. Sea water came from the same source. Page (13) has made a careful analysis of this water for mineral constituents.

The results of the observations are given in Table I. *Nereis virens*, *Laonice viridis*, and *Limulus polyphemus* showed a considerable degree of toleration for sea water diluted to one fourth its normal salinity. *Arabella opalina*, *Glycera dibranchiata*, and *Lepidonotus squamatus* lived for many days in one half sea water and one half fresh. Most of the worms tested lived several days in three fourths sea water. *Nereis* in higher concentrations of sea water climbed out of the dishes at intervals and was found in varying degrees of desiccation, hence some individuals probably died sooner than some of those in more dilute solutions. Every *Laonice* studied lived throughout the period of observation and was active at the end. The Hydroides in tubes did not live as long as those which were removed. This was probably due to the fouling of the water by small organisms in and on the mollusc shells to which the tubes of these worms were attached. The *Limuli* used were small individuals, less than 10 cm. long. All that were tested in solutions as low as one fourth sea water survived to the end of the observations. One individual lived 26 hours in a solution of one eighth sea water, and another lived two hours in fresh water.

Nereids have been observed in various localities to be noteworthy for their ability to endure considerable dilution of sea water (3, 7, 11, 13). In India there is a species of *Limulus* which lives in brackish water (3). Vaughn (24) found that several species of corals survived a reduction of twenty per cent. in the salinity of the sea water and he interpreted this as indicating that the ocean has been in the past less salty than now. The observations described in this paper show that many worms have similar toleration. The writer cannot see that general features of bodily structure and habitat are especially correlated with ability to survive in diluted sea water. Apparently delicate branchiate worms, like *Chætopterus*, *Diopatra*, and *Laonice*,

endure fresh water about as well as apparently tougher worms, such as *Phascolosoma* and *Lumbricillus*. The only oligochæte observed, *Lumbricillus agilis*, was not as hardy as many polychætes when placed in diluted sea water. In low salt concentrations all the animals studied showed a tendency to swell and became turgid and extended. *Laonice* perhaps showed this least; *Lepidonotus* and *Lumbricillus* perhaps most; *Amphitrite* frequently bled and died soon. The individual *Nereis* which lived and was active for twenty-one days in a medium containing only one fourth sea water became very active and soon began to shrink to its normal size when replaced in undiluted sea water.

Table I.

Time in Hours which Animals Lived in Sea Water and Various Dilutions of it.

A indicates that an animal was apparently in good condition when the observations were discontinued.

Name of Animal.	No. Observed.	Sea Water.				
		1	$\frac{3}{4}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$
Amphitrite ornata (Leidy)	12	65	115	7	2	
Arabella opalina (Verrill)	5		302A	302A	.7	
Arenicola marina (L.)	2				2	
Chætopterus pergamentaceus Cuvier	10	403A	403A	4	1	
Cirratulus grandis Verrill	4	475A	475A	8	3	
Clymanella torquata (Leidy)	13	487A	487A	38	.3	
Cystenides gouldii Verrill	3		35	256A	4	
Diopatra cuprea (Bosc)	17	280A	478A	30	.2	
Glycera dibranchiata Ehlers	9	498A	498A	498A	.7	.4
Harmothoe imbricata (L.)	7	228A	228A	4	.5	
Hydroides hexagonus Bosc	12	260A	260A	105	.5	
Laonice viridis (Verrill)	8	230A	230A	230A	230A	
Lepidonotus squamatus (L.)	13	474A	474A	379A	.3	
Limulus polyphemus (L.)	13	191A	191A	192A	192A	40
Lumbricillus agilis Moore	5	8	100	130	.3	
Maldane urceolata (Leidy)	1			8		
Nephthys bucera Ehlers	2	356	115			
Nereis virens Sars	13	236+	259A	500A	500A	3
Phascoloscoma gouldii (Pourtales)	4	252A	298	.3	.2	
Pista palmata (Verrill)	5	303A		35	26	

Summary.

The ability of *Limulus*, *Phascolosoma*, and eighteen marine annelids to survive in various dilutions of sea water was studied. Most of the animals lived for a week or two in a mixture of three

fourths sea water and one fourth fresh; several species lived in one half sea water. *Limulus*, *Laonice*, and *Nereis* lived and were active for periods of two to three weeks in one fourth sea water, but died in weaker solutions.

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THE EFFECT OF THE QUANTITY OF CULTURE MEDIUM ON THE DIVISION RATE OF *OXYTRICHA*.

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The hypothesis advanced by Robertson (1923) that unicellular organisms produce an autocatalytic substance which aids in bringing about their own division, has been subjected to investigation by a number of workers who find little if any evidence supporting Robertson's view. In a recent paper Greenleaf (1926) presents the results of a series of experiments concerned with the effect of the amount of culture medium and cell proximity on the division rates of various Protozoa. From these results he is unable to find any evidence in favor of the autocatalytic effect. On the other hand he finds evidence corroborating the findings of Woodruff (1911), namely, that in small quantities of culture medium the division rate of isolated individuals is retarded. This is due, according to the author, to the accumulation of excretory products and their greater concentration in small amounts of medium. This retardation effect is lessened as the quantity of medium is increased.

These differences in findings leave the problem still open for investigation. It is the purpose of this brief article to set forth the results of some experiments made in an attempt to determine the relation of the amount of culture medium to the division rate of certain protozoa. No attempt has been made at this time to go into all the phases of the whole subject. The experiments were carried on with a species of *Oxytricha*. One of these organisms was isolated from a laboratory aquarium into ten drops of sterile .05 of one per cent. beef extract solution, on a depression slide. This was kept in a moist chamber made of a covered glass disk 7 x 4 x 4 inches. A pure line was established by reisolating every day for several days. When it was desired to start the experiments one or more slides were allowed to stand

48 hours or until there were sufficient organisms in one slide to start the desired number of cultures. All cultures of a given experiment were therefore descendants of a single individual isolated 48–72 hours before.

Oxytricha was chosen after a number of preliminary trials with various ciliates, because of its rapid division rate. At laboratory temperature there are usually two or more divisions in 24 hours and in rare cases as many as six generations have been produced in a day. The large number of individuals produced seemed to make the organism a more favorable subject for these experiments than other more slowly dividing ciliates.

The experiments consisted in comparing the division rate of sister cells in four and ten drops of .05 of one per cent. of sterile beef extract.

In order to keep the organisms in as near the same atmospheric environment as possible slides with two depressions were used. One depression contained the organism in four drops, the other the culture of ten drops. Four slides were used in the earlier experiments, but the number was later increased to ten. The pipettes for measuring the quantity of culture medium were drawn out so that one drop was about one one-hundredth cubic centimeter.

The first series of experiments was carried on at room temperature but as this fluctuated greatly it was decided that controlled temperature conditions were essential for reliable result. For temperatures above ordinary room temperature a Freas electric incubator was used and for temperatures below room temperatures a Cenco water bath was used, in which the moist chambers were sunk almost to their tops in the water and a constant temperature of 18° C. was maintained.

In all cases counts were made at the end of the 24-hour period. One or more slides were set aside to develop "seed" for the next day's experiments. Division rate was measured by the total number of organisms produced in 24 hours from an isolated individual in a given amount of culture medium.

In making the isolations care was taken to take individuals of as near the same size as was possible. In a culture divisions do not occur simultaneously and there are therefore small individuals

—the result of very recent division, large individuals almost ready to divide, and a majority of an intermediate size, probably those full grown. This latter group was selected for the experiments. All organisms used in one experiment were of the same line of protoplasm and as near the same age with respect to the last division as could be determined.

Series I.

Series I. was carried on at laboratory temperature which fluctuated very much from day to night, varying probably from 16° C. to 22° C. The results, however, are fairly constant.

Experiment.	4 Drops.		Experiment.	10 Drops.	
	No. of Cases.	Total No. Individuals.		No. of Cases.	Total No. Individuals.
1.....	4	28	1.....	4	27
2.....	4	10	2.....	4	8
3.....	4	13	3.....	4	13
4.....	4	22	4.....	4	15
5.....	4	24	5.....	4	14
6.....	4	20	6.....	4	16
7.....	4	27	7.....	4	36
8.....	4	45	8.....	4	20
9.....	4	15	9.....	4	14
10.....	4	15	10.....	4	16
	40	219		40	179
	Average, 5.48			Average, 4.47	

This indicates that at room temperature the individuals in four drops have a much higher division rate. The average division rate is 24.6 per cent. higher in the former than in the latter.

Series II.

Series II. was similar to Series I., except for the fact that the temperature was maintained at about 24° C. The division rate was therefore higher than in Series I.

Here again the four drops show a higher division rate than ten drops. The average for four drops is 12.3 per cent. higher than for ten drops.

4 Drops.			10 Drops.		
Experiment.	No. of Cases.	Total No. Individuals.	Experiment.	No. of Cases.	Total No. Individuals.
11.....	4	50	11.....	4	28
12.....	4	21	12.....	4	17
13.....	4	26	13.....	4	27
14.....	4	37	14.....	4	35
15.....	4	38	15.....	4	48
16.....	4	31	16.....	4	28
17.....	4	10	17.....	4	15
18.....	4	76	18.....	4	47
19.....	2	59	19.....	4	82
	34	348		36	327
	Average, 10.2			Average, 9.08	

Series III.

Since the difference in division rate in the two given amounts was larger in the lower (room) temperature (Series I.) it seemed advisable to compare the differences in rates at different controlled temperatures. Therefore, a series of experiments was run with individuals grown in four and in ten drops at 18° C. and at 23° C. The organisms used in any one day were from the same isolated individual. They were presumably as near the same age as it was possible to get them and all had been under identical environmental conditions.

18° C.						23° C.					
4 Drops.			10 Drops.			4 Drops.			10 Drops.		
Exp.	No. Cases.	Individ.	Exp.	No. Cases.	Individ.	Exp.	No. Cases.	Individ.	Exp.	No. Cases.	Individ.
B	10	19	B	9	15	A	7	41	A	10	45
D	9	38	D	10	32	C	10	59	C	10	77
F	10	32	F	10	31	E	10	83	E	10	80
H	10	46	H	10	36	G	10	103	G	10	82
L	10	44	L	10	34	K	10	138	K	10	142
N	10	26	N	10	24	M	10	87	N	9	60
P	9	36	P	10	29	O	10	97	O	10	76
	68	241		69	201		67	608		69	562
	Average, 3.54			Average, 2.91			Average, 9.07			Average, 8.1	

As in Series I. and II. the division rate in Series III. is higher in four drops than in ten drops. At the lower temperature the difference in the division rate in the two groups is greater than it is at the higher temperature. In the former the organisms in four drops have a higher rate by 21.64 per cent. while in the latter the four drops have a higher rate by 10.19 per cent.

In all of these experiments the division rate in four drops exceeds that in ten drops by more than ten per cent. If we consider only the amounts of culture medium and disregard temperature the results are as follows:

4 Drops.			10 Drops.		
Series.	No. of Cases.	Individ.	Series.	No. of Cases.	Individ.
I.....	40	219	I.....	40	179
II.....	34	348	II.....	36	327
III 18°....	68	241	III 18°....	69	201
23°....	67	608	23°....	69	562
	209	1,416		214	1,269
	Average, 6.77			Average, 5.93	

The division rate in four drops is considerably higher than that in ten drops—a difference of 14.17 per cent. This result is perhaps more significant than the others since there are over 200 cases considered.

The results of these experiments have a direct bearing on two important problems. The first of these is the question of the autocatalase. Robertson (1923) quite definitely showed that with the infusorian *Enchelys*, isolated in a limited amount of culture medium, there was an increasing reproductive rate during the second twenty-four hours of a culture over that of the first twenty-four hours. He also found that, within certain limits, division rate of *Enchelys* was higher in the first twenty-four hours in small quantities of culture medium than in larger quantities and that individuals isolated into one cubic centimeter or more of medium rarely survive.

In his interpretation of these and other experiments Robertson was forced to the conclusion that "the only possible inference

that can be drawn from this phenomenon is that infusoria discharge into the culture medium some substance which accelerates their own multiplication."

As further test of this theory he showed that reproduction would occur in sterile culture medium or in distilled water and cites the experiments of Peters (1921) with *Colpidium* to show that bacteria as food are not essential for reproduction of infusoria if the proper ingredients necessary for growth are put into the medium.

The second question for consideration is that concerning the effect of the excretory products on the division rate of protozoa. Woodruff (1911) has shown that the rate of division is very markedly lowered by the accumulation of excretory products. Greenleaf (1926) has taken up the problem and using several different infusorians arrived at the same conclusions as those reached by Woodruff fifteen years before. These results are in direct contradiction to those of Robertson and Greenleaf concludes that as far as the organisms with which he worked are concerned there is no evidence of the autocatalytic effect. He further concludes that infusoria tend to multiply faster in larger amounts of culture medium and that one individual in a given amount of medium multiplies faster than each does when two individuals are in the same amount. This difference is explained on the basis that two organisms would raise the concentration of the excretory products to a degree injurious to themselves more rapidly than a single individual would do.

The results of the experiments upon which this paper is based seem to favor the idea advanced by Robertson. In all series in which four and ten drops of culture medium were used the average division rate in four drops was higher than in ten drops. Since the culture medium was sterile when the organisms were introduced, the question of food does not seem to be one of prime importance in our consideration. The most plausible explanation therefore is that *Oxytricha* produces a substance which is liberated into the surrounding medium and which reacts on the infusorians to bring about cell division, and that the concentration of the substance necessary for bringing about cell division is reached in the smaller amount of culture medium before it is in the larger amounts.

The conflicting conclusions reached by various workers leave the question still unsolved. It is possible that the various results have been due to the fact that organisms with a high division rate show the effect of the autocatylitic substance more readily than do organisms which divide only once or possibly twice in twenty-four hours. Robertson used *Enchelys* which divides many times a day. Woodruff and Greenleaf used larger infusorians which have a much slower rate of division. Between these extremes *Oxytricha* has an intermediate division rate and in the experiments cited undoubtedly shows a higher average rate of division in the smaller amounts of culture medium. There seems to be no explanation of this so satisfactory as that suggested by Robertson for *Enchelys*, namely, that *Oxytricha* produces a substance which reacts on the organism to bring about division, and that the concentration of this substance is reached in sufficient quantities to bring about cell division in small quantities of culture medium before it is in greater quantities.

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CONJUGATION, DIVISION, AND ENCYSTMENT IN *PLEUROTRICHA LANCEOLATA*.[1]

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Introduction.

Evidence has been steadily accumulating of recent years that many closely related species differ from each other in number of chromosomes, the numbers often being multiples, or multiples of some common factor. Because of this fact and because the processes of conjugation have been thoroughly studied in only a very few hypotrichs, the present investigation was undertaken. *Oxytricha fallax* and *Pleurotricha lanceolata*, although placed in different genera, are morphologically quite similar, and it was thought that a comparison of the two species with respect to chromosome number and details of conjugation would be of interest. It has been shown before in at least one case (*Chilodon uncinatus*, MacDougall, 1925) that new species may arise de novo from old ones among the protozoa, the chief differences being in chromosome number, and it is not unlikely therefore that this often happens in nature. A study of division and encystment was undertaken as a natural corollary.

Pleurotricha lanceolata and Similar Species.

Pleurotricha lanceolata was first described accurately by Stein in 1859, although two other species which he regards as the same had been described previously—*Stylonychia lanceolata* and *Keratium calvitium*, the former by Ehrenberg in 1832 and the latter by Müller. But Ehrenberg's organism had 16 to 18 cirri on the dorsal surface as well as the full complement on the

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ventral surface, and Müller failed to see the abdominal cirri, so that priority of description and name belongs to Stein.

Pleurotricha lanceolata was also described and figured by Kent (1880 to 1882), but his description is slightly different from that of Stein. In addition to the double row of marginal cirri on the left-hand side, Stein shows a very short third row in the middle region of the body which Kent omits, as well as the anterior third of the inner marginal row.

The race which I have used (Fig. 1) fits Kent's description very closely, but is only about half as large, and the inner marginal row of cirri is found only in the middle third of the body. Stein gives the size as varying between 83 and 143 μ, and Kent makes it just about twice as great. I have found the average size to be about 140 μ, the extremes being 100 to 165 μ, if exconjugants which are always exceptionally small are omitted.

Oxytricha bifaria (Stokes, 1887) also resembles *Pleurotricha lanceolata*, differing principally in having only a single marginal row of cirri extending around the body, and in having eight instead of six cirri on the anterior quarter.

There is also a considerable resemblance between *Oxytricha fallax* and the chief species under discussion. The former has five anal cirri arranged in an oblique row, the two posterior extending only slightly beyond the body margin.

Material and Methods.

The cultures were started from a single individual obtained from a mass culture of leaves and swamp water, which in turn was secured from Orient Springs, near Amherst, Massachusetts, November 5, 1925. These cultures were continued until May 1927, the medium being hay infusion, to which in some cases a small amount of peptone was added.

All preparations were fixed with Calkins' modification of Schaudinn's fluid, and stained with hemotoxylin, both the long and short methods being used.

Division.

The first indication of division is seen in the macronuclei, in each of which a nuclear cleft, or kernspalt, appears at the extreme

outer ends, and this gradually moves across the nuclei to finally disappear at the inner ends. Simultaneously, or very shortly afterward, a new adoral zone begins to form in the midregion of the body. This stage of division is shown in Fig. 2.

Very soon the micronuclei begin to enlarge and a clear space, or halo, appears about each. At about the same time a division center appears and divides, the two products remaining connected by a centrodesmose (Figs. 3 and 4). I have never been able to detect this endosome in the resting micronucleus however, and it is difficult to observe even in division. As the dividing nucleus enlarges it elongates, and the chromatin appears to be arranged in very fine strands, some of which seem to be in reality rows of granules (Fig. 4 *A*). At this time it stains very faintly. The chromatin next appears to become more or less concentrated at the poles (Fig. 4 *B*). As all this goes on the macronuclei change their shape, becoming almost round and the kernspalt finally passes off the inner ends (Figs. 4, 5, 6). The new adoral zone meanwhile increases in size, and new cirri may be seen developing beside the old ones of the old adoral zone (Fig. 6). In Fig. 5 the new ventral cirri may also be seen close to the adoral zones, where they first arise. As the time approaches for final separation of the daughter individuals these cirri gradually move to more nearly their normal positions (Fig. 11).

In the meantime the chromatin of the micronuclei appears to condense into discrete masses, which take the form of threads of varying length (Fig. 5 *B*). These gradually combine, until the nucleus appears to be composed of closely packed and more or less tangled threads, as in Figs. 5 *A* and 6 *B*, and finally a definite spindle appears (Fig. 6 *A*). The poles of the latter move apart and a very characteristic figure is produced which is shown in Fig. 7 *C*. Here the chromosomes appear as heavily staining Vs, the limbs of which are connected by heavy and deeply staining strands. Apparently division is really longitudinal, the two daughter chromosomes taking on a V shape and appearing connected at the ends for a while thereafter. Their final separation is shown in Fig. 7 *A*. Fig. 8 *A* shows a somewhat later stage of the metaphase.

At about this time the macronuclei begin to elongate (Fig. 8) and the remaining stages in division are passed through very rapidly, the time required being about fifteen minutes at ordinary temperatures, as compared to more than an hour for the preceding steps. The micronuclei enter the anaphase, which is quickly completed, requiring only a very few minutes, and which may be seen in Figs. 9, 9 *A*, 9 *B*, 9 *C*. The telophase is shown in Figs. 10, 11, 11 *A*, 11 *B*, and 12. The macronuclei divide at this time (Figs. 11 and 12), and the parent animal very shortly separates into two daughter individuals (Fig. 13).

Discussion.

The process of division in ciliates is so well-known that few comments are necessary, since different species differ only in minor details, and fission in *Pleurotricha lanceolata* follows the general plan. There are, however, a few points in which this species differs from others, and in other respects there are interesting resemblances.

The presence of a kernspalt is said to be common to all hypotrichous ciliates, even during the major part of the resting stage (Calkins, 1919), but I have never been able to see it in *Pleurotricha* except when the animal was about to divide, as shown by the presence of a rudimentary adoral zone. This happens even before there is any visible change in the micronuclei.

Apparently the early appearance of a new cytostome with its accompanying peristomial apparatus is characteristic of most dividing infusoria, *e.g. Uroleptus mobilis* (Calkins, 1919), *Chilodon uncinatus* (MacDougall, 1925), although there are other cases in which its development is more delayed as in *Paramœcium trichium* (Wenrich, 1926).

It seems to be generally accepted that in division there is a complete reorganization of the entire cell, extending even to the disappearance and subsequent reformation of all the organelles, including trichites in organisms which have them, undulating membranes, cirri, and even cilia. According to Wallengren (1900) the cirri of the old adoral zone in hypotrichous ciliates are gradually absorbed as new ones take their places, and I have

been able to confirm this observation for *Pleurotricha*. That this reorganization does not always extend to the old oral apparatus, however, is indicated by Wenrich (1926) who was unable to find evidence that the old cytostome disappeared and was reformed in *Paramœcium trichium*.

It is a remarkable fact that all the cirri, with the exception of the marginal ones, arise in the immediate neighborhood of the adoral zones, and indeed the first indications of their appearance are visible very shortly after the adoral zone itself appears. That this was the case in the Oxytrichidæ was noticed by Sterki (1878), and has been noted for other groups by other investigators. MacDougall (1925) found that new cilia in a dividing *Chilodon uncinatus* first appear near the new pharyngeal baskets.

The presence of a centrosome which divides as an initial step in division has until recently not been noted in ciliates. It was reported by Calkins in the first maturation division of *Uroleptus mobilis* however (Calkins, 1919), although he evidently did not see it in ordinary division, and MacDougall found it in *Chilodon uncinatus*. To this list can now be added *Paramœcium trichium* (Wenrich, 1926), and *Pleurotricha lanceolata*.

Encystment.

Encystment is a common occurrence in the life cycle of *Pleurotricha bifaria*, although what the conditions are which determine its occurrence I have been unable to discover. It may occur in old cultures, and is perhaps most frequent under those circumstances, but it also occurs in pedigreed drop cultures, which are changed every day, and in which the division rate is rapid. Of four individuals which have arisen by division from a single ancestor during the preceding twenty-four hours it is not uncommon to find one or two encysted, while the others continue to divide actively.

The account of nuclear phenomena which follows is admittedly inadequate, but it is given since no other description has been made of this stage in the life cycle of this particular species, and because the history of the cytological changes which occur in ciliates during encystment is notoriously incomplete.

The first change appearing in an individual about to encyst is

like that occurring in other infusoria under similar circumstances. The shape becomes spherical, the contractile vacuole gradually ceases to pulsate, the food vacuoles and all the organelles disappear (Fig. 14). The next step is the secretion of the cyst wall, shown in Fig. 15. It is thick, quite pliable but very tough, and without visible openings. The outside is covered with irregular, short spines. Such a cyst is shown by Stein (1859) for *Pleurotricha lanceolata*, but Cienkowsky (1855), who gives three figures of the cyst formation of *Stylonychia lanceolata*, does not show the spines. Nevertheless these two species are regarded by Stein as the same, as already stated.

Soon (probably under normal conditions in about twenty-four hours) the old macronuclei, and apparently one of the micronuclei, are bodily extruded through the cyst wall, although the opening through which they pass is never visible under any other circumstances (Fig. 16). This leaves the encysted animal in the condition shown in Fig. 17. The remaining nucleus then proceeds to divide but I am unable to say much as to the details of this division. Apparently some of the micronuclei shown in Figs. 18 and 19 are in process of division, and the process appears to be a typical mitosis. As to how a cyst in the condition of that in Fig. 19, just referred to, reaches that shown in Fig. 20 I am unable to say. Cysts are exceedingly difficult to stain and destain at this stage.

Figs. 20 and 21 show the final stages in the reorganization. As may be seen there has been a great decrease in the quantity of cytoplasm, and a corresponding decrease in chromatin. Animals at this stage may be seen swimming about within the cyst, but I have never been able to get them to leave the cyst, despite washing, prolonged observation in cultures in which the media was changed daily, and attempts to rupture the cyst wall artificially. The latter always failed because of the tough pliable nature of the wall. Both distilled water, tap water and Ringer's solution were used for washing.

Moore (1924) found that *Spathidium spathula* behaved in a similar fashion, though she was more successful in liberating encysted individuals by puncturing the cyst wall. Under ordinary circumstances few encysted animals would excyst, and

drying, which Calkins (1919) found necessary to induce the excystment of *Uroleptus*, and the addition of fresh medium, found efficacious by Moody (1912) in securing the excystation of *Spathidium*, both proved unsuccessful.

But the cytological changes which occur during the encystment of *Spathidium spathula* appear to be markedly different from those which take place in *Pleurotricha*. There is no extrusion of nuclear or cytoplasmic material from the cyst in Spathidium, but the macronuclei degenerate and finally disappear, the fragments being absorbed by the cytoplasm. The micronuclei remain practically unchanged. Shortly before redifferentiation occurs, preparatory to leaving the cyst, new macronuclear anlagen appear which strongly resemble those formed in the late stages of conjugation. It is possible that more study would show that the new macronuclei arise in a similar way in *Pleurotricha*, but I have seen no indication of it in any of my preparations.

That an extensive nuclear reorganization takes place in many infusoria during encystment has been known for a long time, but I have found no mention of the extrusion of nuclear material in any other species, although Prowazek (1899) believed that such a phenomenon occurred during conjugation. Fermor (1913) found that in *Stylonychia* there was a fusion of the two micronuclei previous to the formation of a new nuclear apparatus, and it is possible that the same thing occurs in encysted individuals of *Pleurotricha*. But in one instance I have actually observed one of the two micronuclei being extruded along with the macronuclei.

Conjugation.

During the eighteen months in which the cultures were maintained conjugation occurred but rarely, and epidemics of it were never observed. Even in mass cultures containing thousands of individuals it was usually necessary to look for an hour or more to obtain a few pairs. Because of the small number of conjugants no sections were made and all the results herein described were obtained from whole mounts, fixed with Calkins' modification of Schaudinn's fluid, stained with hematoxylin, and mounted individually. Various methods of inducing conjugation have been

suggested by investigators who have found them more or less useful with certain species, and a number of these methods were tried in this case, but with practically no success.

Conjugating individuals fuse by the adoral surfaces (Fig. 22), the entire peristome of one member of the pair disappearing completely. There seems to be no reason why it cannot function in the other conjugant but there is very little evidence that it does so, for food vacuoles become fewer in number as conjugation progresses, and exconjugants are always small. The time from fusion to final separation varies from eighteen hours to five days, but the usual duration is twenty-four hours.

Behavior of the Micronuclei.

The micronuclei normally go through three maturation divisions, only two taking part in each. The others degenerate more or less rapidly, although some may persist even after the interchange. The pronucleus usually undergoes two cleavage divisions. Of the four products one enlarges and eventually gives rise to the macronuclei of the reorganized exconjugant, one degenerates, and the remaining two form micronuclei.

The First Maturation Division.

This division requires more time than any of the others—at least eight hours—and is also strikingly different in type. The micronuclei at first show no change but soon increase in size, become surrounded by a clear space, or "halo," and stain more faintly than usual. The chromatin takes on a finely-granular appearance. Shortly an endosome, or division center, appears, and in favorable preparations two may be seen (Fig. 28 *A*, *C*), but I have never been able to detect an intradesmose connecting them, although in vegetative division and in the other divisions of conjugation it can often be seen. This division center increases in size until it becomes hemispherical and stains very heavily (Fig. 28 *C*, *D*). In the meantime spindle fibers begin to appear, and the spindle takes on the typical parachute appearance (Fig. 28 *C*, *D*, *E*). The chromatin in the expanded top of the parachute, which is at first composed of very small, dimly staining granules, condenses into larger granules which stain

more heavily and these pair to form heavily staining chromosomes of dumbbell shape. These granules are altogether too numerous to count, and in most preparations this is also true of the chromosomes, but I have made counts in a few cases which will be discussed at greater length below. The chromosomes now move to the center of the spindle and divide longitudinally (Fig. 28 *F*, *G*). Stages of the anaphase and telophase are shown in Figs. 28 *H* to *M*. The most characteristic feature of this division, aside from the dumbbell shape of the chromosomes, is the way in which they lag and the peculiar curve at the poles of the spindle.

The Second Maturation Division.

This follows rapidly on the first, and is over in much less time, with the incidental result that material showing it is difficult to obtain. It is also of very different character. The micronuclei which are to divide enter the prophase almost before the telophase of the preceding division is complete (Fig. 32 *A*). At this stage they stain faintly, and rows of granules appear which are arranged in a more or less "whorled" fashion (Fig. 32 *B*). Fig. 32 *C* represents a stage which is seldom seen, but it is presumably earlier than those shown in the two preceding figures. The nucleus next enlarges and the rows of granules become threads (Fig. 32 *D*). This may well be in reality a leptotene stage, since reduction occurs during this division. There is soon evidence of a definite spindle which is at first of a peculiar oval shape (Fig. 32 *E*, *F*). I have been unable to get preparations showing the metaphase and later stages of the anaphase, but the early anaphase is shown in Fig. 32 *F*. I have made a number of counts of the number of chromosomes concerned in this division, the average result being forty. Two stages of the telophase are shown in Fig. 32 *G* and *H*.

The Third Maturation Division.

The third division differs in type from both the preceding, and requires more time than the one just described. The micronuclei which are to divide enlarge, and become very finely granular. I am uncertain as to whether there is an intradesmose

connecting the products of the division of the intranuclear endosome, but I believe that there is. What is apparently one end of it can be seen in Fig. 36 *A*. These granules now condense to form rows, and the latter in turn become much coiled chromosomes which often appear double (Fig. 36 *B*, *C*). The metaphase is shown in Fig. 36 *D*, and is considerably like that of ordinary vegetative division. The chromosomes in both the second and third maturation divisions are rod-shaped, in contrast to the dumbbell shape which they have in the first, and the V and irregular rod shape of the cleavage divisions. The long-pointed anaphases are characteristic of the third division, and long drawn-out telophase (Figs. 36 *E* to *I*).

The interchange is a rapid process, and the wandering nuclei do not appear to differ in any respect from the stationary ones. New adoral zones appear at this time or very early during the first cleavage division (Fig. 37).

The First Cleavage Division.

The amphinucleus is shown in Fig. 40, and is apparently divided into two parts, doubtless representing the two pronuclei. The stages in the first cleavage division are shown in Figs. 41 *A* to *H*. The nucleus, which increases very much in size shortly after fusion, becomes smaller, and forms a peculiar sort of spindle (Fig. 41 *A*), in which the chromatin appears to be condensed into a heavy ribbon, twisted more or less upon itself, and quite definitely double. Chromosomes soon appear which are arranged in bouquet fashion, about a small endosome, and which at this stage seem to be, in some cases at least, in pairs (Fig. 41 *B*). They then straighten out and form a characteristic spindle which has at first two definite parts, possibly representing the two pronuclei (Fig. 41 *C*). At this time the chromosomes are definitely double, and apparently twisted about each other. The metaphase is figured in 41 *D*, and the anaphase soon follows. Apparently the chromosomes in the later stages of this division are shaped like very acute Vs. Figs. 41 *E* to *H* show the anaphase and telophase. The former is rather characteristic in its early stage because the chromosomes are so widely separated within the receding plates.

The Second Cleavage Division.

The first cleavage division which has just been described is a rapid process, but the second one is much slower. A division center appears, as in two of the three maturation divisions, and divides, the products remaining connected by an intradesmose (Fig. 46 *A*). The nucleus then becomes elliptical, and the chromatin becomes arranged in long deeply-staining strands which are at first quite regular in arrangement (Fig. 46 *B* and *C*). These elongate and become twisted (Fig. 46 *D*, *E*), some of them appearing double. The whole spindle resembles nothing so much as a tangled skein of yarn at this stage, and possibly the stage represented in Fig. 46 *D* is in reality a spireme. The strands in the following figure are definitely polarized and probably are really chromosomes. The metaphase is shown in Fig. 46 *H*. It does not differ very much from that in the first cleavage division. The anaphase is very peculiar, as can be seen from Figs. 46 *J* to *M*. At the poles of the spindle a "cap" of very deeply-staining chromatin is formed and the chromosomes lose the definite shape which they had in the metaphase, apparently coalescing into more or less irregular masses which take a very heavy stain. These are very characteristic and make the later stages of the second cleavage division differ from all the others. The strands persist for some time in the anaphase (Fig. 46 *L*, *M*).

The Occasional Third Cleavage Division.

Following the second division there may in some cases be another, but when it occurs only two of the four nuclei take part as a rule, so that exconjugants in which this third division has taken place have six nuclei, rarely eight. All the stages of this division which I have observed are like corresponding ones of the second division.

The Behavior of the Macronuclei.

The anterior of the two macronuclei elongates during the maturation divisions, and may occasionally divide by a process of mass division, but only in very rare cases. At the same time it undergoes a slow fragmentation, so that the cytoplasm usually

has a number of heavily staining masses of chromatin at this time, which often simulate degenerating micronuclei derived from previous divisions. The posterior macronucleus may elongate slightly, but I have never observed it to divide and it undergoes much less fragmentation than the other.

The chromatin fragments and degenerating micronuclei usually disappear about the time of the interchange, or soon afterward, but may in some cases persist until after the cleavage divisions.

Reorganization of the Exconjugant.

Reorganization begins before separation, and usually requires several days. One of the four nuclei resulting from the second cleavage division becomes very large and coarsely granular, and at the same time loses much of its capacity to stain with hemotoxylin. It is destined to form the macronuclei of the reorganized exconjugant and is shown in Figs. 47, 48, 49, 50, 52 and 53. This nucleus divides at the first division of the exconjugant, and then divides again without corresponding division of its possessor, thus restoring the normal macronuclear condition (Fig. 55).

But it is apparently possible for reorganization to become complete without prior division of the exconjugant, and an individual in which this has happened is shown in Fig. 54. It may be that this can happen in individuals in which there has been a third cleavage division.

The micronuclei of the reorganized conjugant are derived directly from two of the three which remain from the second cleavage division. The third persists for a time but eventually degenerates. As to what happens when there have been three cleavage divisions I am unable to say, since I have been unable to observe enough cases.

The remains of the old macronuclei persist for a day or two as more or less circular, deeply staining and vesicular masses of chromatin (Figs. 49 to 52), but they eventually disappear completely, leaving no trace (Fig. 53).

The Number of Chromosomes.

In order to determine the number of chromosomes a large number of counts were made in several different stages, par-

ticularly from an especially favorable preparation showing the early anaphase of the first maturation division, and from several preparations of the anaphase of the third maturation division.

Since the total number of chromosomes is so large in the anaphase of the first maturation division accurate counts are difficult, but ten were made as a check for the other counts described below. The mean of these ten counts was eighty-six, which would indicate that the diploid number is forty-three. The standard deviation was found to be 15.4, the coefficient of variation 17.9, and the probable error $\pm$ 3.46. There is no doubt therefore that the counts are very significant.

A few counts were made of the chromosomes in the early anaphase of the second maturation division, and the average was close to forty, again indicating therefore that this figure is close to the diploid number.

Since the most favorable preparations for chromosome counting were those of the third maturation division these were given the most study. To insure results as free from error as possible seventy counts were made from several preparations showing the anaphase of this division. A curve was constructed of these counts and the mode found to be 19. The mean was 19.62, and the probable error $\pm$ .295. The coefficient of variation was 18.6 per cent., and the standard deviation 3.66. It is therefore certain that the counts are highly significant, and there is no doubt that the haploid number of chromosomes is close to twenty, with a high degree of probability that it is exactly that number, making the diploid number forty.

Since the number of chromosomes in *Oxytricha fallax* is twenty-four (Gregory, 1923), there is therefore no obvious relation as far as number of chromosomes is concerned between this species and *Pleurotricha lanceolata*.

Discussion.

Although only a relatively small number of ciliates have been studied with reference to the phenomena of conjugation the process appears to be essentially the same in all. From his own observations Maupas (1888) divided it into eight phases—*A*, the period of preparation preceding the first meiotic division; *B*,

the first division; *C*, the second division; *D*, the third division; *E*, the interchange and fusion of the pronuclei; *F*, the first cleavage division; *G*, the second cleavage division; and *H*, the period of reorganization preceding the first fission of the exconjugant.

These phases have been shown to hold for all infusoria so far studied, including *Pleurotricha lanceolata* which is the subject of this paper, although in a few forms such as the Vorticellidæ, Ophryoscolecidæ, and *Euplotes patella*, there are one or more preliminary divisions before the meiotic divisions begin. (In the case of the Vorticellidæ this happens only in the case of the microgamete).

Ciliates as far as at present known fall into two classes according to the behavior of the micronuclei in the first phase—those which undergo a prophase like *Paramœcium*, the micronucleus being drawn out into a crescent, and those in which a parachute or candelabra-like figure is formed, to use Calkins' term. To the last group belong *Onychodromus grandis* (Maupas, 1888), *Bursaria truncatella* (Prowazek, 1899), *Didinium nasutum* (Prandtl, 1906), *Anoplophrya branchiarum* (Collin, 1909), *Uroleptus mobilis* (Calkins, 1919), *Oxytricha fallax* (Gregory, 1923) and *Chilodon uncinatus* (MacDougall, 1925). The character of the first division, already described, makes it necessary to add *Pleurotricha lanceolata* to this list.

As might perhaps be expected from the close morphological resemblance of *Oxytricha fallax* and *Pleurotricha lanceolata* the details of the conjugation process are much alike, and while they are also similar to those which occur in *Uroleptus mobilis* as described by Calkins, the resemblance is much less striking.

The formation of the parachute preliminary to the first maturation division more nearly resembles the corresponding stages in *Uroleptus* than in *Oxytricha*, but differs from both. In the latter, although the parachute fibers are focused on a single granule derived by division from the endosome just as they are in *Pleurotricha*, there is also a centrodesmose connecting the two halves of the endosome which I have never been able to observe in *Pleurotricha*. In *Oxytricha* there appears to be no endosome, and the place of the basal granule is taken by a row of granules.

The chromosomes in this division appear to be formed by the fusion of the granules into which the chromatin is divided early in the prophase, but whether the number of these granules bears any constant relation to the number of chromosomes, as Gregory believes it does in the case of *Oxytricha*, I am unable to determine. These chromosomes which at first make up the top of the parachute appear to move down the fibers until they reach the center of the spindle when they form an equatorial plate. The other pole of the spindle appears to be formed by one of the two halves of the endosome which remains in the top of the parachute, the other as already stated, forming the granule at its base. This is apparently the same as in *Uroleptus*.

The number of nuclei taking part in any of the three maturation divisions is apparently never greater than two in the case of *Pleurotricha*, although forms in which there are three micronuclei occur. Of the four products of the first division all but two degenerate, and the same thing happens after each of the other two maturation divisions. The number of nuclei taking part in each maturation division in *Oxytricha* appears to be variable, although there are always at least two. In other multinucleate ciliates which have been studied there is considerable variation in this respect. In *Bursaria* all the sixteen or more nuclei may take part in the first meiotic division (Prowazek, 1899), and in *Uroleptus* there may be two, three or four primary spindles (Calkins, 1919), but these are exceptions.

The second maturation division is apparently of different character from the first in most ciliates, and lacks the elaborate preliminaries of the latter, with the result that it requires much less time. The micronuclei in most cases do not return to the resting stage between the first and second division, although there are exceptions (*e.g.*, *Chilodon*, MacDougall, 1925). In all these respects therefore *Pleurotricha lanceolata* is typical.

In nearly all cases in which a careful study has been made reduction has been found to take place in the second meiotic division, although the first division was thought to be the reducing division in *Paramœcium caudatum* (Calkins and Cull, 1907), and in *Oxytricha fallax* (Gregory, 1923). But Dehorne (1920) regards the third division as the one in which reduction occurs in the

former form. In the case of *Pleurotricha* there is no doubt but that it occurs in the second, for the following reasons: The total number of chromosomes taking part in the first division in the beginning anaphase has been determined to be in the neighborhood of eighty, as already stated, whereas there are only half as many concerned in the second division, and the average number moving toward each pole of the third maturation spindle has been determined, as stated above, to be about twenty.

The only protozoa in which reduction is known to occur at any other time (with the two exceptions already mentioned), are *Diplocystis* (Jamieson, 1920), and *Aggregata eberthi* (Dobell, 1915). It is suggested by Dobell and Jamieson that this may be a universal occurrence among the Telosporidia.

The third maturation division and the interchange take place exactly as in *Oxytricha*, and are very much alike, even to details in the form of the spindle. Nothing is said as to the existence of a division center or intradesmose however. The dumbbell character of the chromosomes is not lost until the third division in the case of *Oxytricha*, and it is stated that the chromatin streams toward the poles in the form of granules. I find that the chromosomes are definitely rod-shaped in *Pleurotricha*, in both the second and third divisions, although the rods are heavier and shorter in the former, and may give place to rows of granules in the late anaphase stages of the latter. The dumbbell character is never regained after the first of the three maturation divisions.

The two cleavage divisions follow the usual course, again resembling closely those in *Oxytricha*, although not many details are given in Gregory's paper. The products of the second cleavage division are equal as in *Oxytricha*, but differ in this respect from the state of things in *Uroleptus mobilis*, in which one of the two nuclei derived from the first division gives rise to the macronuclei and the other to the micronuclei of the reorganized exconjugant (Calkins, 1919). I have found no other case in which the anaphase of the second cleavage division resembled that in *Pleurotricha* however, and it differs strikingly from that in *Oxytricha*.

The occasional third cleavage division is also a striking charac-

teristic, and as far as I can discover it occurs in no other hypotrich so far described, although it is admitted that the number studied is thus far small. In other forms such as *Paramœcium caudatum* (Calkins and Cull, 1907), and several of the Vorticellidæ, a third division of the amphinucleus occurs, but as a regular thing. In *Bursaria* (Prowazek, 1899), there are four divisions before differentiation occurs.

The behavior of the macronuclei in *Pleurotricha* follows the usual rule—degeneration eventually occurs, although it is not completed until after separation. But division of the macronuclei, although apparently the rule in *Oxytricha*, is very rare in *Pleurotricha*.

The time required for reorganization and the details of the process differ considerably in different cases, but the process as it occurs in *Pleurotricha* is not significantly different from the essential features of reorganization in other species. Prowazek believed that the remnants of the old macronuclei must be extruded bodily from the cell in *Stylonychia pustulata* and *Bursaria truncatella* (1899), but I have seen no evidence of this in the present instance, nor has it ever been observed by anyone else. He based his hypothesis on the fact that nucleins are indigestible.

The Physiological Effect of Conjugation.

The significance of conjugation has been much disputed, one school holding that it is the necessary result of senescence in any given race, and that by it the death of the race could be averted and rejuvenation, expressed chiefly in an acceleration of the division rate, be secured. Maupas held this view and since his time Calkins has been its leading exponent. Another group has regarded conjugation as a process useful chiefly as a means of producing variations, which are much greater and much more frequent in exconjugants than in ordinary vegetatively reproducing individuals. Jennings has been one of the chief expounders of this view.

Many experiments have been tried to settle this question and since the results have differed considerably for different species I have endeavored to find out the effect of conjugation in *Pleuro-*

tricha lanceolata, as measured by the division rate of exconjugants when compared to closely related non-conjugating lines.

The experiment consisted in isolating one hundred exconjugants, and following them daily as long as possible. Of this number 92 per cent. died without division, usually about four days after separation, and of the remaining eight only one gave any evidence whatever of rejuvenation. The others divided two or three times and then all died. This single individual gave rise to a line which divided between two and three times a day for six days, and from then until the death of the line four weeks later, the average daily division rate gradually declined until during the last two weeks it was much less than one per day.

The controls during this period averaged from one to two divisions a day, and showed no evidence of senescence for the six months for which pedigreed cultures were maintained. Both controls and exconjugants were in the same media.

It is realized that one hundred cases is scarcely enough to draw final conclusions from, and yet the percentage of deaths is so high that it does not seem likely that a greater number of cases would have given significantly different results.

Jennings (1913) in a long and comprehensive series of experiments demonstrated that with *Paramœcium* "Conjugation decreases the rate of fission, causes a great increase in variation in fission rate, brings about many abnormalities, and greatly increases the death rate," but Mast (1917), using *Didinium nasutum*, was unable to secure evidence of any such effect. He found that there was no appreciable effect on either death rate, fission rate, or increase in variation of fission rate, thus proving however that there was also no rejuvenation.

Calkins, in a long series of experiments with *Uroleptus mobilis*, has shown that in this species at least conjugation appears to have a genuine rejuvenating effect (Calkins, 1919 and 1926), and Woodruff and Spencer (1924) conclude that conjugation has a similar effect in the case of *Spathidium spathula*, although they prefer the term "high survival value" to that of "rejuvenation."

It has been shown by many investigators working with various species that as culture methods are improved the longevity of cultures without conjugation can often be indefinitely increased,

so that it can probably be safely concluded that conjugation is at least not a necessary process if environmental conditions are sufficiently favorable.

Since cultures of *Pleurotricha* have been maintained for eighteen months with very little conjugation (none in the pedigreed lines which were carried for six months), and since in the cases mentioned above none of the exconjugants gave rise to lines which continued for any length of time, although kept under identically the same conditions as controls which maintained a uniform and fairly high division rate, it can be concluded I think that conjugation is not an indispensable part of the life cycle. This conclusion is supported by Baitsell (1914) who carried on experiments of this nature with both *Pleurotricha lanceolata* and *Oxytricha fallax*.

It is nevertheless apparent that a process which is so universal among infusoria as conjugation must serve some useful purpose, and that in nature exconjugants do not always die. Why then do they die in cultures? It is impossible to give a definite answer, but it has been suggested that media which is suitable for active vegetative multiplication may not always be suitable for conjugation and exconjugants, and this may well be the explanation.

Summary.

1. *Pleurotricha lanceolata* is a hypotrichous ciliate belonging to the family Pleurotrichidæ. The species has as its chief characteristic six anal cirri, divided into two groups. The anterior of the two groups consists of four cirri—three very large ones arranged in an oblique row, and a smaller one, a little forward of the posterior end of the row. The second group includes two cirri, both of them very long and projecting well beyond the posterior end of the animal. The usual size of vegetative individuals is 140 μ.

2. The process of division does not differ particularly from that in other infusoria, except that in the very early stages an endosome appears and divides, the products remaining connected by an intradesmose. This has been described in *Paramœcium trichium* but in few if any other species. The process of division is initiated by the appearance of a rudimentary adoral zone and

a kernspalt in each of the macronuclei. The micronuclei divide by typical mesomitosis. When division of the latter is virtually complete the macronuclei follow suit, dividing amitotically, and then the cell itself divides. All organelles appear to be regenerated, the old ones being absorbed.

3. Encystment may occur at any time, and appears to bear no relation to periods of depression, to division or to conjugation. The old macronuclei are extruded bodily from the cell, and a single micronucleus remains. It is uncertain whether the other is always extruded with the macronuclei or fuses with the first micronucleus. An uncertain number of micronuclei are formed from the single remaining micronucleus, and from these the normal nuclear complex is rebuilt, the process being complete at the time the animal is ready to leave the cyst.

4. The nuclear changes which occur during conjugation are essentially the same as those described for other ciliates. There are three maturation divisions, an interchange of pronuclei, and two or rarely three cleavage divisions. The four products of the second division are at first alike, but one soon enlarges and eventually gives rise to the new macronuclei of the reorganized exconjugant. One of the others degenerates and the other two form the new micronuclei. The old macronuclei degenerate after separation of the exconjugants. There may or may not be one division of the latter before reorganization is completed.

5. Reduction occurs in the second maturation division. The diploid number of chromosomes is forty, as nearly as can be determined, and the haploid number twenty. The chromosomes are dumbbell-shaped in the first maturation division, and rod-shaped in the second and third. They are also rod-shaped in the cleavage divisions, but the shape then is unlike that in the maturation divisions or in vegetative division.

6. Each of the divisions in conjugation differs from all the rest and from vegetative division.

7. Conjugation occurs but rarely in the race of *Pleurotricha* used, and under the conditions of culture virtually always results in the death of the conjugants a few days after separation. In only one instance out of a hundred did it result in anything like rejuvenation, and even in this case the daughter race died within a month.

8. Although the details of conjugation are much alike in *Oxytricha fallax* and *Pleurotricha lanceolata* there is no obvious relation between the number of chromosomes of these two very similar species.

Conclusion.

The cytology of conjugation and division in *Pleurotricha lanceolata* has been described in detail, together with some of the cytological phenomena of encystment. The processes of division do not differ particularly from those which are known to occur among ciliates in general, with the exception of the dividing endosome and connecting intradesmose. Certain features in the division of the micronucleus are strikingly like some which occur in the mitosis of metazoan tissue cells.

A remarkable feature of encystment is the extrusion of the old macronuclei, and perhaps one of the micronuclei, through the cyst wall. After this has happened the normal nuclear complex is rebuilt from the single micronucleus remaining. Whether the presence of the latter is regularly due to the extrusion of the other micronucleus from the cell, or to the fusion of the two original micronuclei is not altogether certain.

The cytological changes of conjugation are in general similar to those previously described in other ciliates, and especially resemble those which occur in *Oxytricha faliax*, but there are important differences in detail. Reduction is shown to occur in the second maturation division. The diploid number of chromosomes is too great to determine exactly, but is probably forty. There may be a third cleavage division in addition to the two which regularly occur, and in this respect *Pleurotricha lanceolata* differs from any other hypotrich so far described.

Conjugation appears to be not only an unnecessary part of the life cycle of this species, at least as long as environmental conditions remain favorable, but is a very dangerous event, for 92 per cent. of one hundred exconjugants died without further division, and only 1 per cent. showed any indication of an accelerated fission rate.

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EXPLANATION OF THE FIGURES.

Magnification (unless otherwise stated) × 550; spindles × 1700.

Plate I.

Early Stages of Division.

Fig. 1. A typical vegetative individual. Occasionally individuals are found with only one micronucleus, or with three.

Fig. 2. The first stage in division. A kernspalt appears at the outer end of each macronucleus, and the beginning of a new adoral zone can be made out (a. z.).

Fig. 3. The micronuclei show an endosome, or division center, in process of division, the halves being connected by an intradesmose. The kernspalt has moved nearer the center of each macronucleus

Fig. 4. The micronuclei have become somewhat larger, and the macronuclei have become almost round. The kernspalt is almost ready to pass off the latter entirely, and the new adoral zone is more conspicuous.

Fig. 4 *A*. An elongated, faintly staining micronucleus; seen in very early division.

Fig. 4 *B*. Same as above, but a little later.

Fig. 5. The chromatin in the micronuclei has condensed into definite threads. The macronuclei have become almost completely spherical and the new adoral zone is well developed. New cirri are making their appearance in the neighborhood of both adoral zones. Enlarged figures of the micronuclei are given under 5 *A* and *B*.

Fig. 6. The anterior micronucleus, shown enlarged in Fig. 6 *A*, has formed a definite spindle.

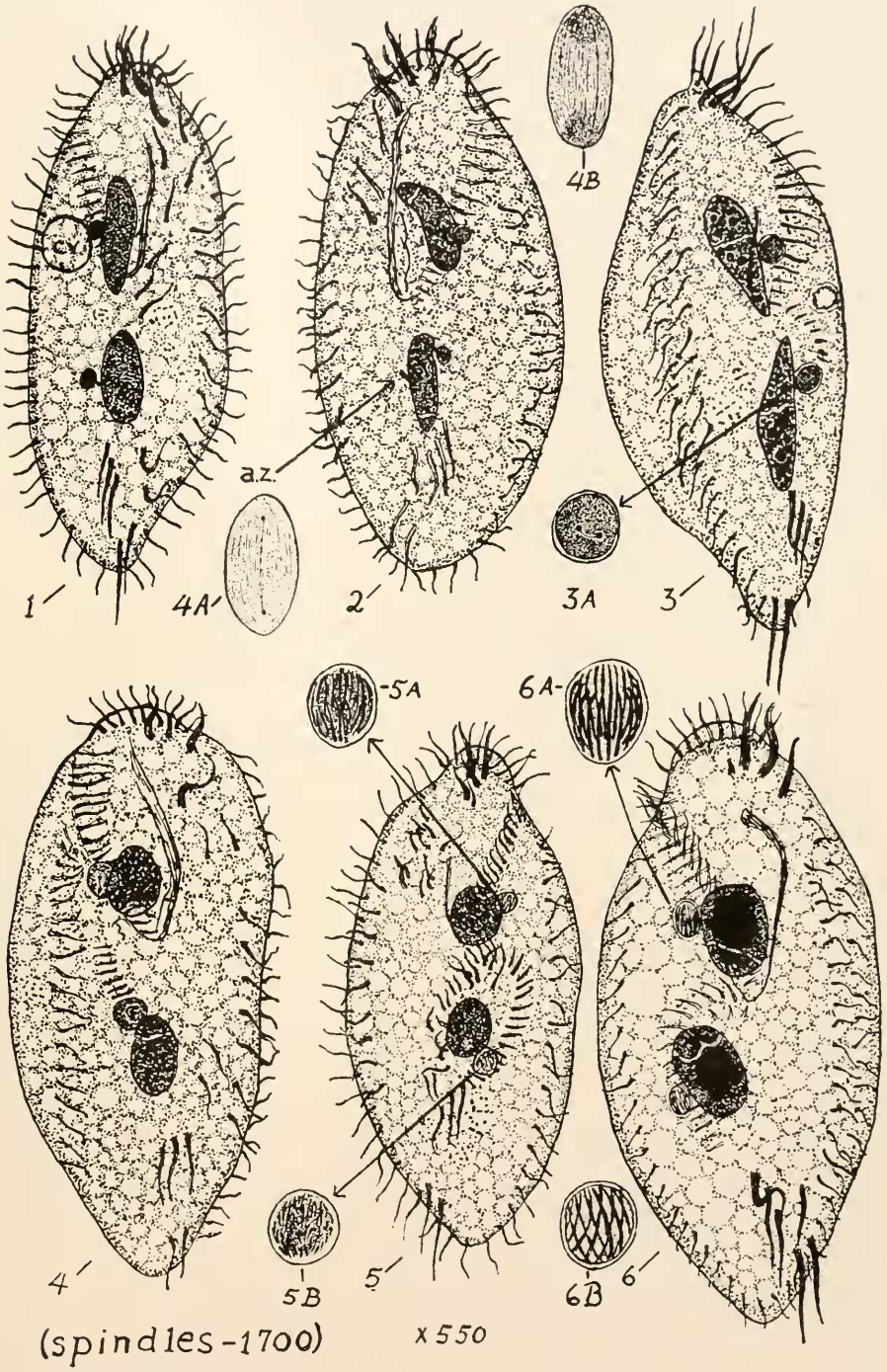

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Plate II.

Later Stages of Division.

Fig. 7. Both micronuclei have formed spindles, which are shown enlarged in 7 *A* and 7 *B*. The cirri are larger, and the macronuclei still almost round.

Fig. 7 *C*. A spindle, magnified about 3500 times, showing the shape of the chromosomes, and the way in which they divide. The heavy strands connecting them are to be noted particularly.

Fig. 8. An individual with but one micronucleus, which is in the metaphase. The macronucleus have elongated, and the new cirri are conspicuous and large.

Fig. 9. An individual in which the micronuclei are in the anaphase.

Fig. 9 *C*. A later stage in the anaphase.

Fig. 10. A still later anaphase.

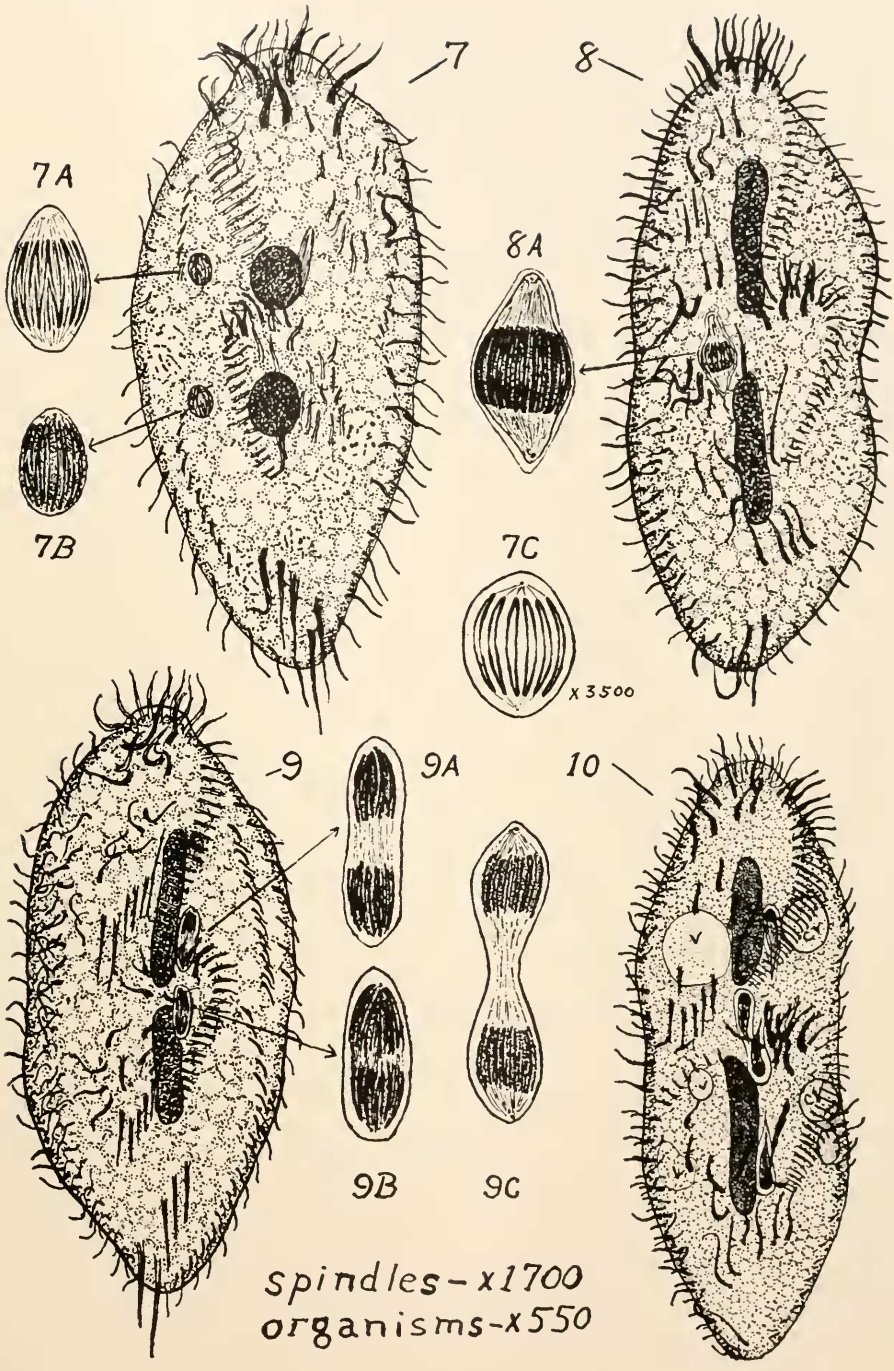
7
8
7A
8A
7B
7C
x 3500
9
9A
10
9B
9C
spindles - x1700
organisms - x550

Plate III.

Final Stages of Division; Cysts.

Fig. 11. The macronuclei are beginning to divide, and some of the old cirri have apparently disappeared. The new ones, originally formed near the adoral zones, are approaching their normal positions.

Fig. 11 *A*. A late stage in the telophase, enlarged from Fig. 11.

Fig. 11 *B*. An earlier telophase.

Fig. 12. The final stage of division.

Fig. 13. An individual which has just divided. The shape differs somewhat from that of a typical vegetative individual, and the anal cirri are not yet quite in their normal positions.

Fig. 14. An early stage of encystment, in which the individual has rounded up, but some of the cirri still remain, and the contractile vacuole is still functional. × 750.

Fig. 15. An encysted animal, in which the cyst wall has just been secreted. × 750.

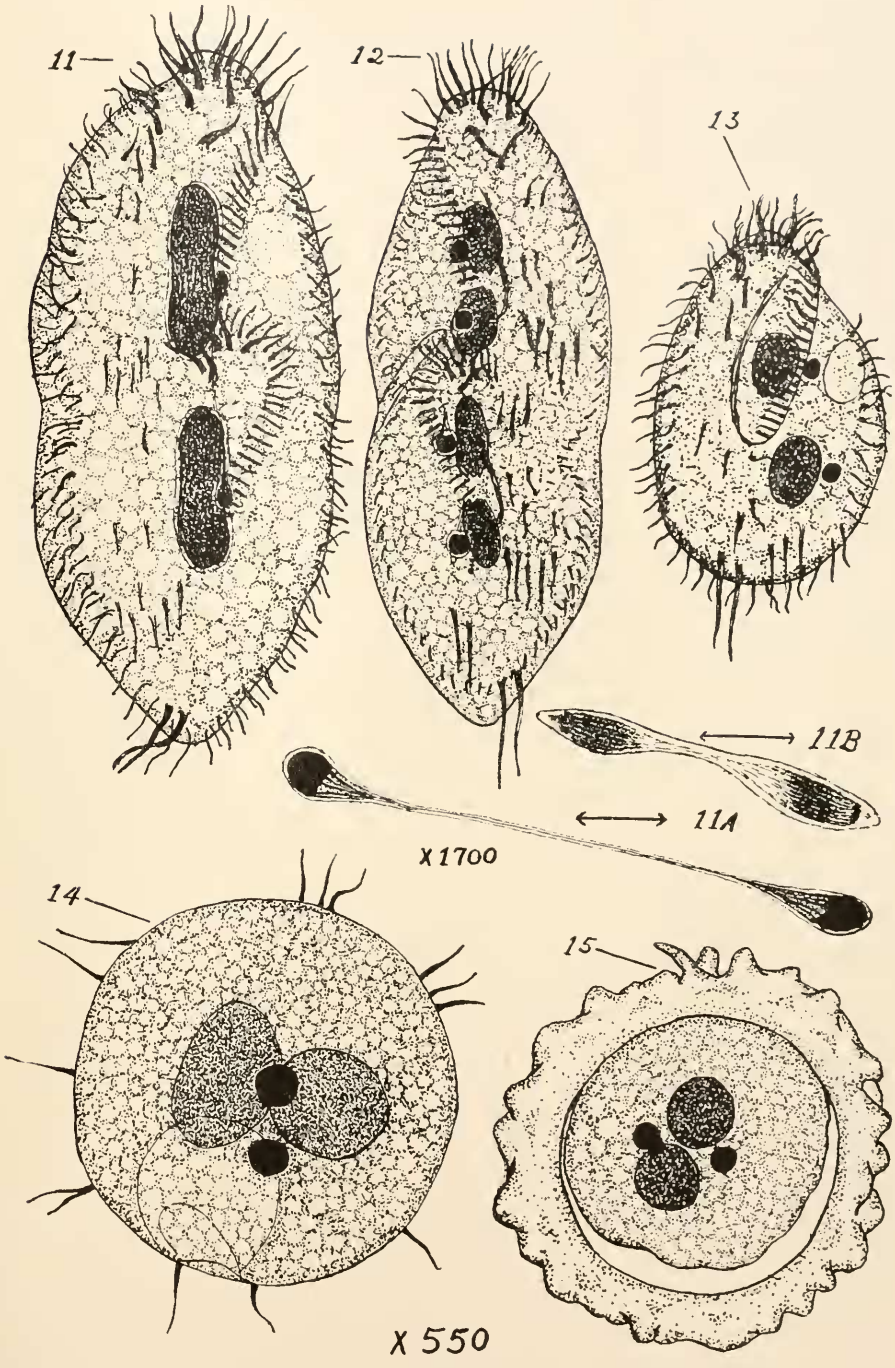

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Plate IV.

Cysts in Various Stages.

Fig. 16. Showing the extrusion of the macronuclei and a small portion of the cytoplasm.

Fig. 17. An encysted animal in which only one micronucleus remains. × 750.

Fig. 18. A cyst containing three nuclei and some darkly staining material which is however not chromatin. × 750.

Fig. 19. A cyst containing seven nuclei, several of which appear to be in mitosis. × 750.

Fig. 20. A cyst in which reorganization is almost complete. × 750.

Fig. 21. Cyst containing a completely reorganized animal. × 750.

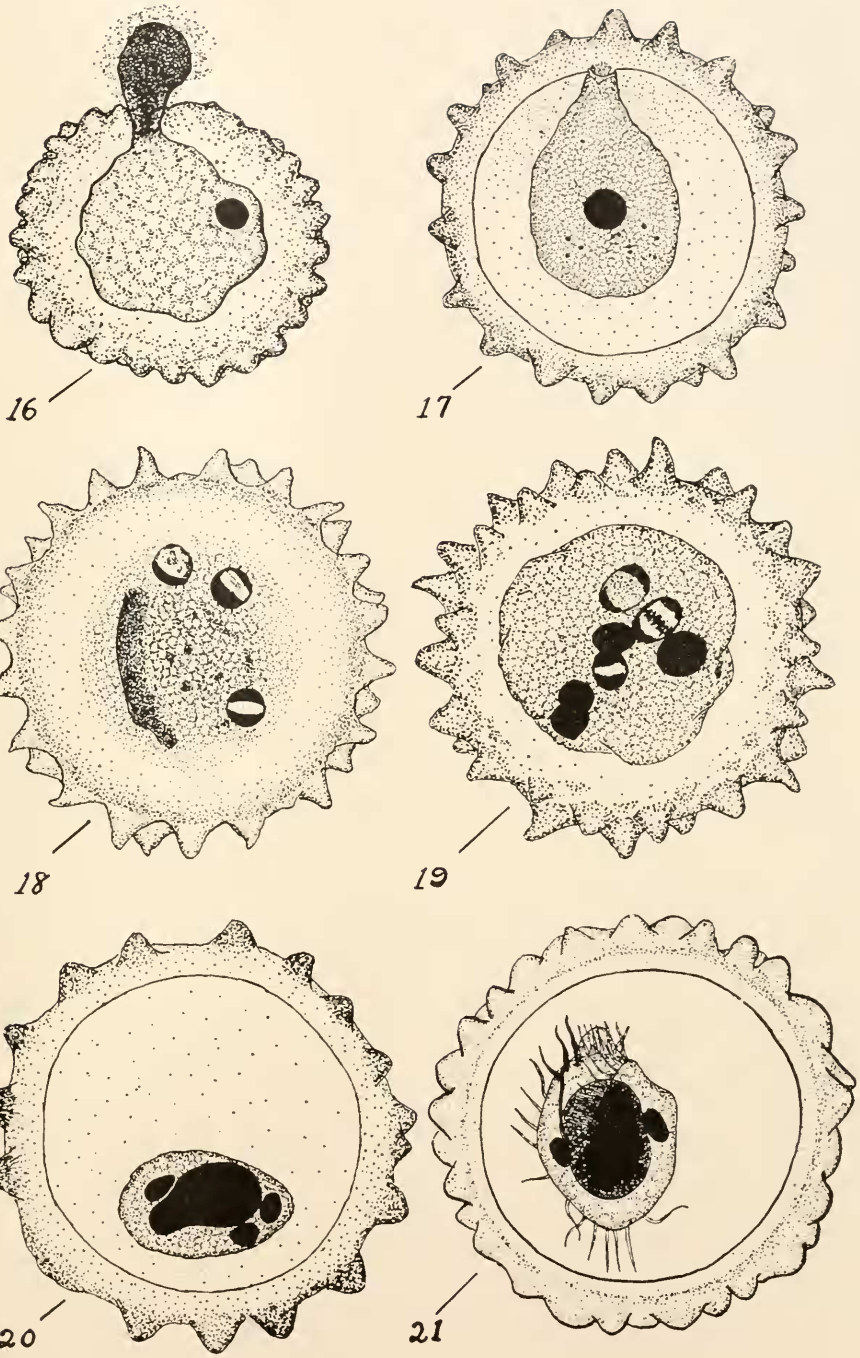

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PLATE V.

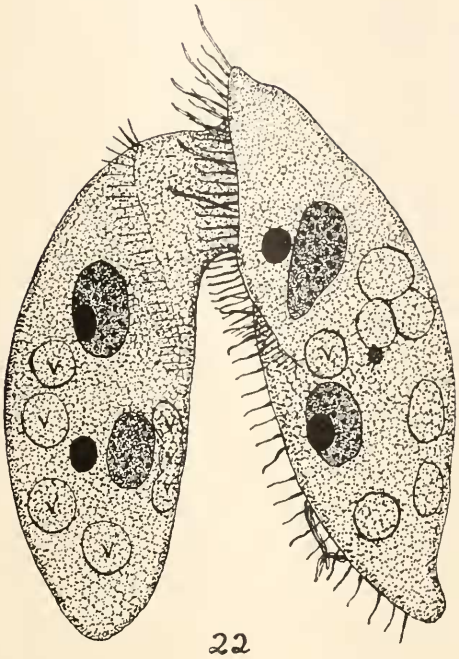
First Maturation Division.

FIG. 22. Initial stage in conjugation, showing the manner of fusion.

FIG. 23. A pair in which the micronuclei have begun to enlarge, preparatory to the first maturation division.

FIG. 24. Here the division centers have made their appearance, and in one nucleus the spindle fibers are becoming visible. Enlarged in 28 *B* and 28 *C*.

FIG. 25. Parachute stage, just prior to the formation of chromosomes. Shown enlarged in Fig. 28 *E*.



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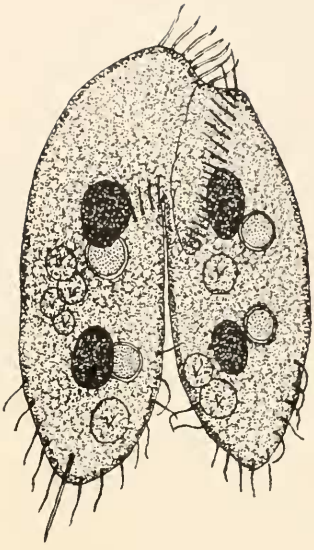

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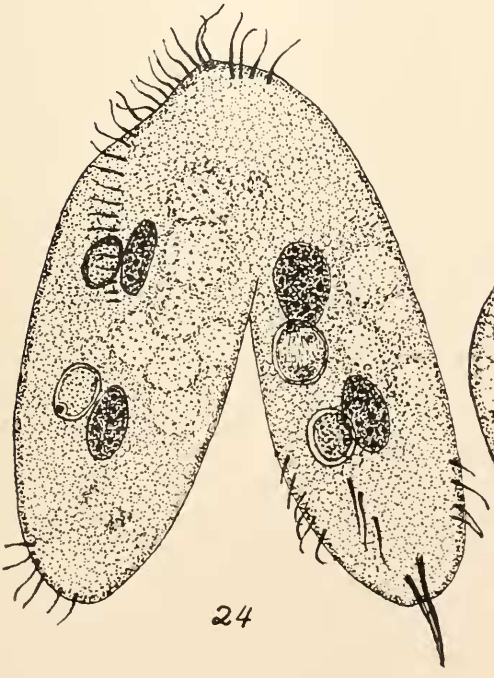

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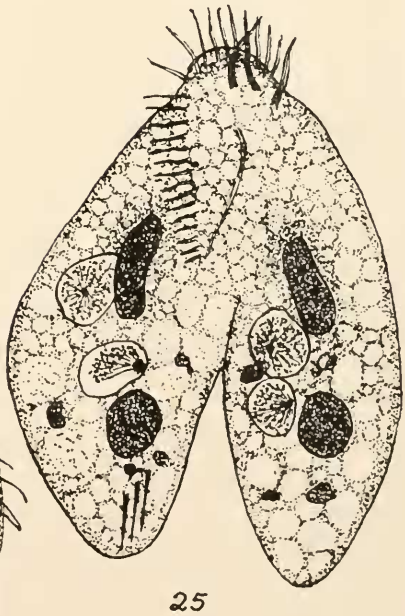

25

PLATE VI.

First Maturation Division.

FIG. 26. Here the micronuclei have entered the anaphase. Two of the spindles are shown enlarged in Figs. 28 *H* and 28 *K*.

FIG. 27. A pair in which a telophase may be seen in each member.

FIG. 28 *A–M*. Various stages of the first maturation division arranged consecutively.

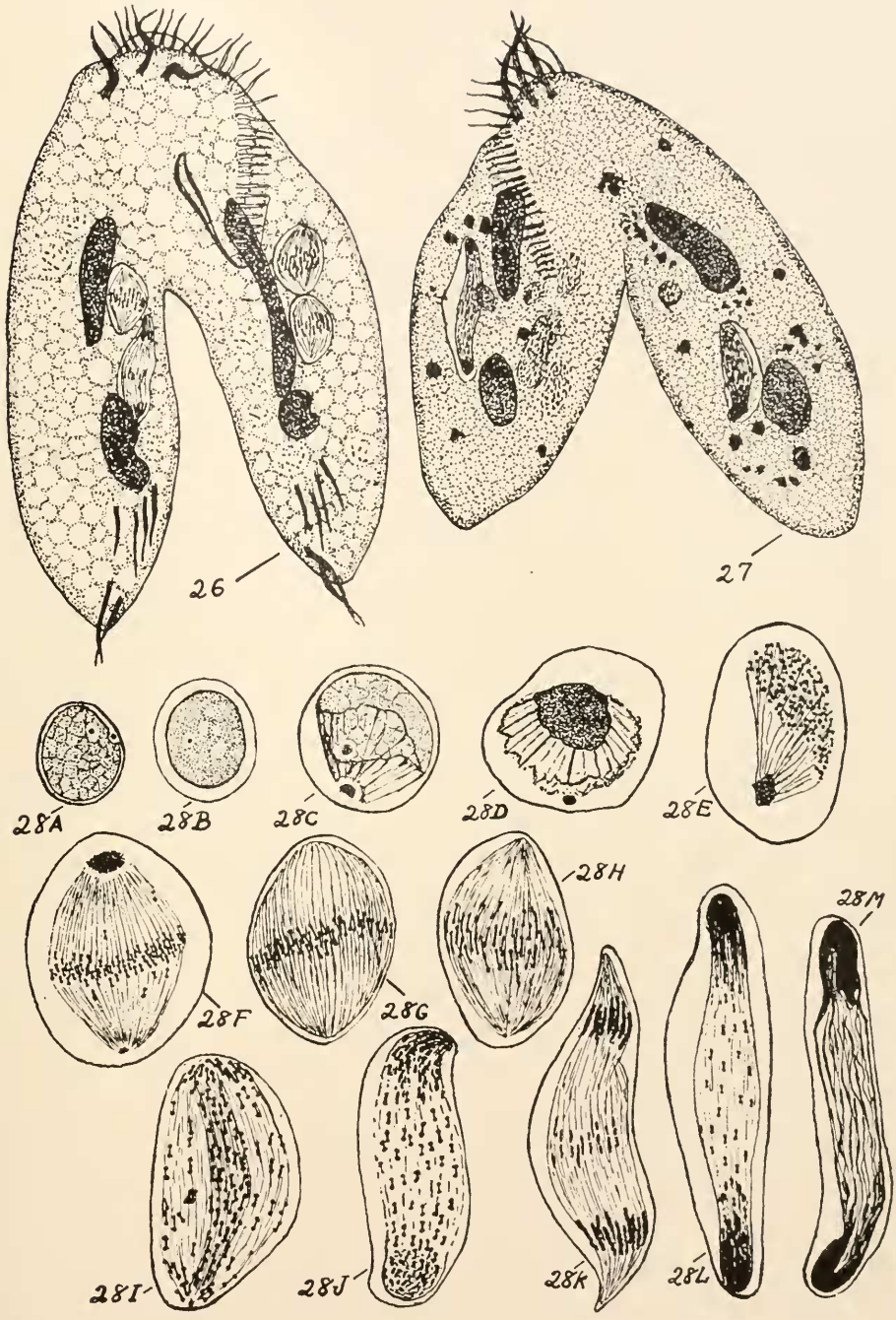

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Plate VII.

Second Maturation Division.

Fig. 29. The left-hand member of the pair shows the final stage in the telophase of the second maturation division. On the right there is a prophase and an early anaphase.

Fig. 30. Each individual of this pair shows a telophase of the second maturation division, and a prophase of the third division is also to be seen in the left-hand member.

Fig. 31. Two prophases of the second maturation division are visible in the left-hand member and a prophase of the third division may be seen on the right.

Fig. 32 *A–H*. Various stages in the second maturation division. "*A*" shows both products of the first maturation division, one of which is already in the prophase of the following division. "*C*" is also a prophase, and should probably precede "*A*."

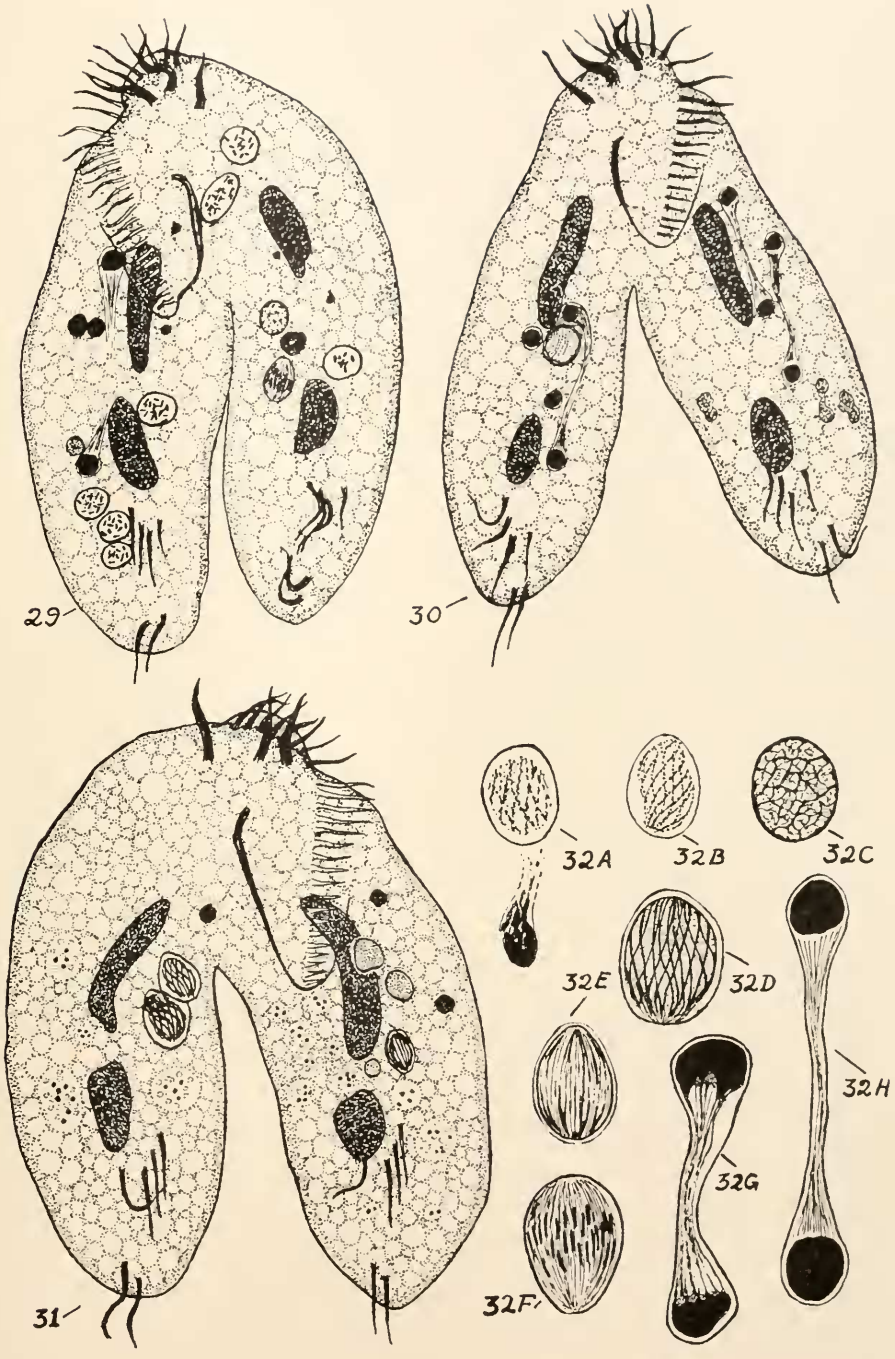

29
30
31
32A
32B
32C
32D
32E
32F
32G
32H

Plate VIII.

Third Maturation Division.

Fig. 33. A pair undergoing the third maturation division. Two anaphases appear on the left and a prophase and anaphase on the right.

Fig. 34. Each member of the pair shows a metaphase, and in addition there is an anaphase in the left-hand member and a prophase in the one on the right.

Fig. 35. A metaphase and anaphase appear on the left, and a metaphase and telophase on the right.

Fig. 36 *A–I*. Stages of the third division, arranged consecutively.

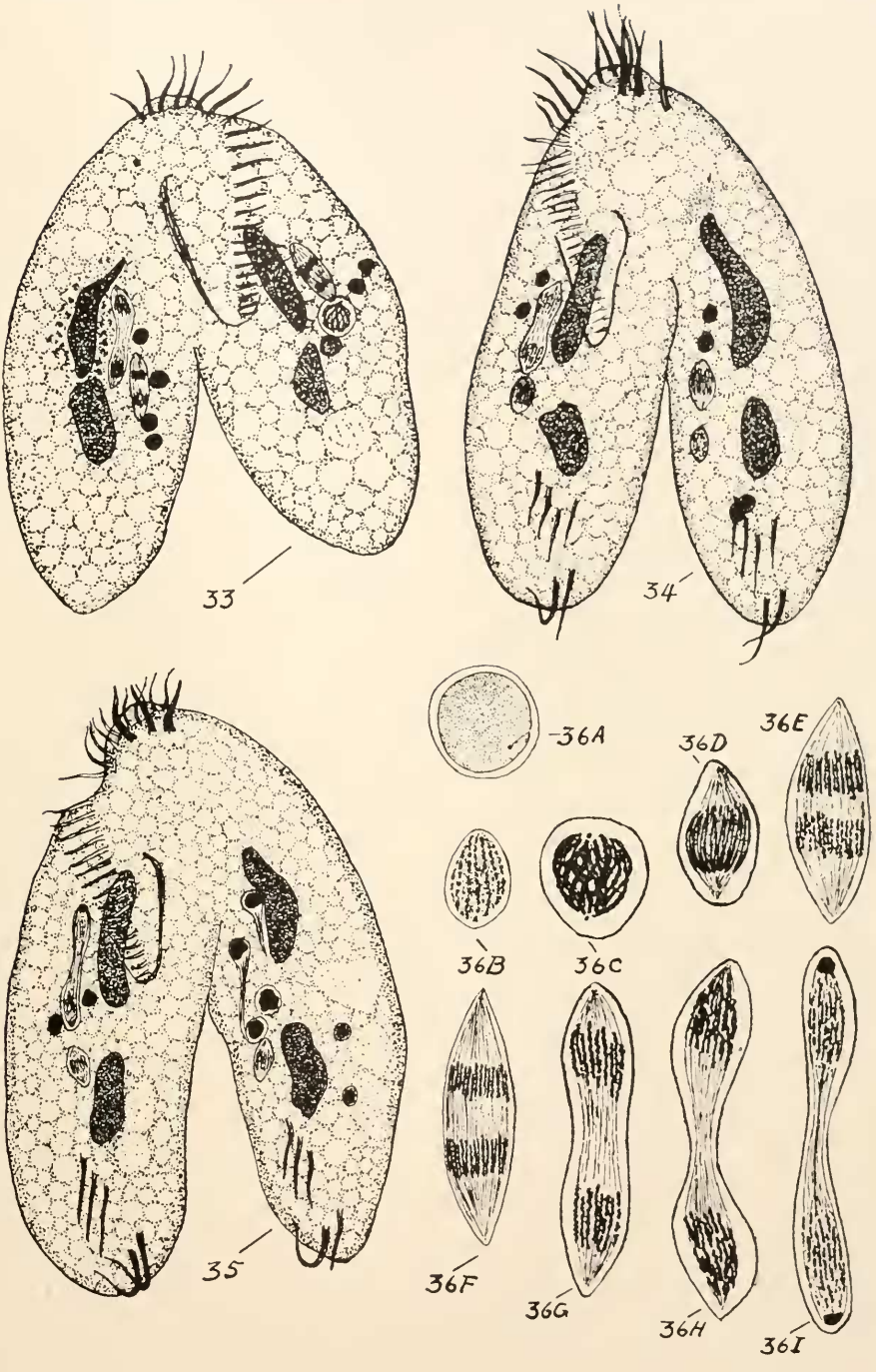

33
34
35
36A
36B
36C
36D
36E
36F
36G
36H
36I

Plate IX.

Interchange and First Cleavage.

Fig. 37. The interchange. The wandering nuclei have already migrated but have not yet fused. Shown as large and relatively faintly staining bodies. New adoral zones are beginning to form.

Fig. 38. The left-hand member of this pair shows the fusion nucleus.

Fig. 39. In the left-hand individual the pronucleus has not yet entered the prophase of the first cleavage division, but division in the right-hand member is well advanced.

Fig. 40. The pronucleus of Fig. 38, enlarged × 1700.

Fig. 41 *A–H*. Stages of the first cleavage division, arranged consecutively.

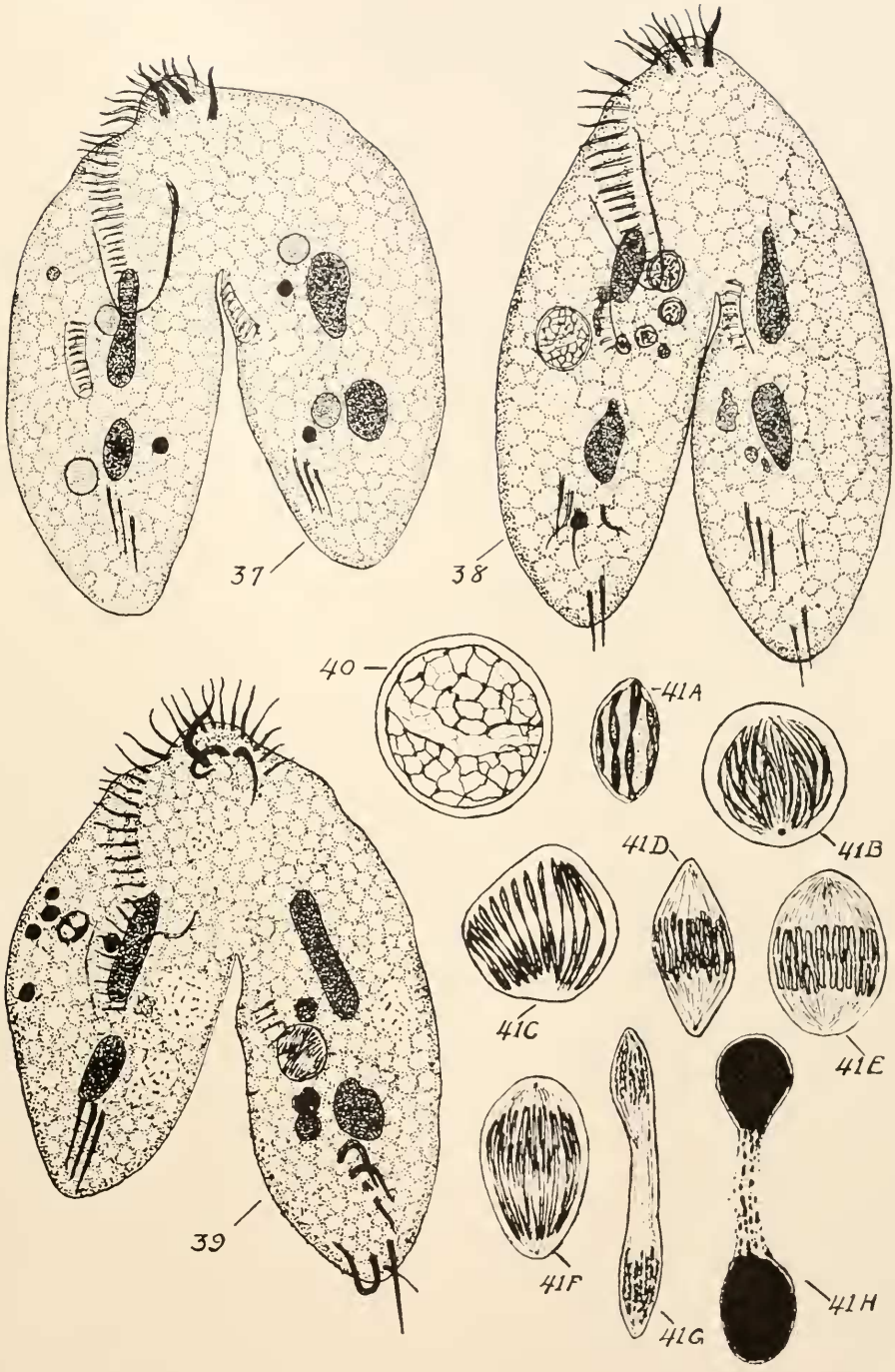

37
38
40
41A
41B
41C
41D
41E
41F
41G
41H
39

PLATE X.

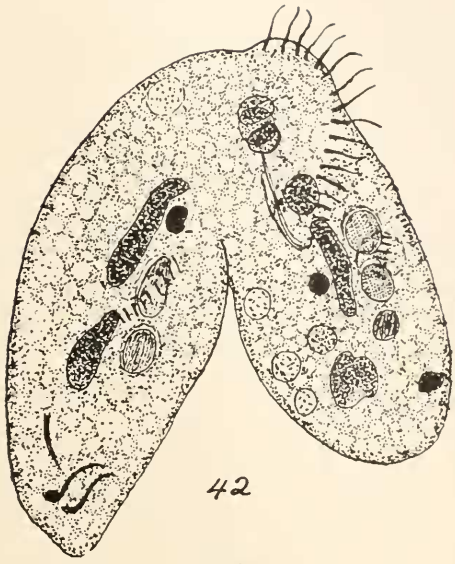
The Second Cleavage Division.

FIG. 42. Shows several prophases.

FIG. 43. In this pair a prophase and anaphase are visible in the left-hand individual, and a prophase and telophase in the right.

FIG. 44. A prophase and telophase may be seen in each of the members of this pair, and also several degenerating micronuclei which still persist from the maturation divisions.

FIG. 45. The cleavage divisions are complete in the left-hand individual but a prophase of the second division is still to be seen in the other member.



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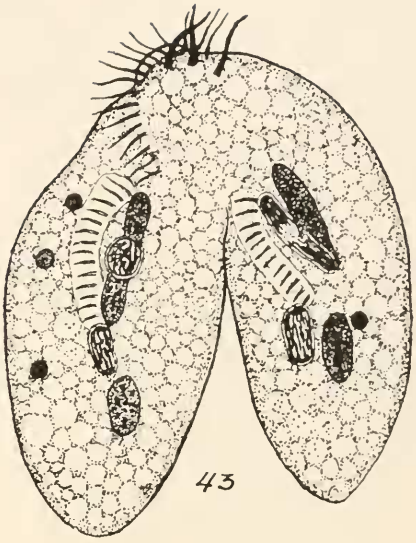

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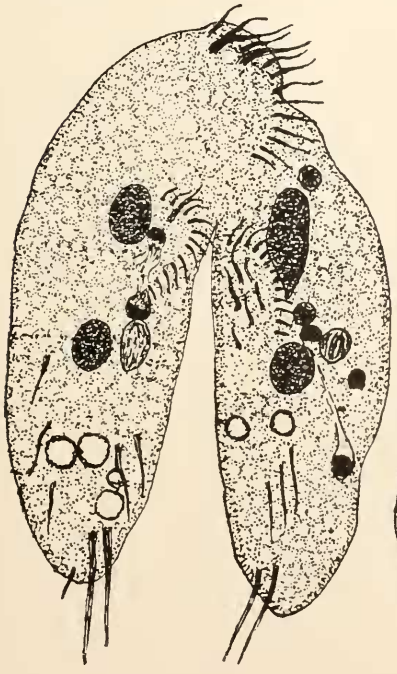

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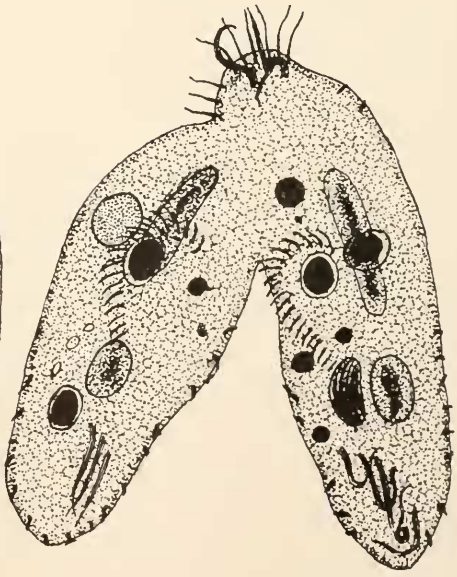

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PLATE XI.

The Second Cleavage Division and Early Stages of Reorganization.

FIG. 46 *A–M*. Stages of the second cleavage division, arranged consecutively.

FIG. 47. Shortly after the conclusion of the last cleavage division. Each individual contains several micronuclei, one of which is increasing in size and will give rise to the macronuclei of the reorganized exconjugant.

FIG. 48. The stage is similar to that in the preceding figure, except that the old macronuclei are becoming vesicular and are beginning to stain much more heavily than normally.

FIG. 49. A pair more completely fused than usual, and with the distribution of micronuclei irregular. The macronuclei are noticeably degenerating.

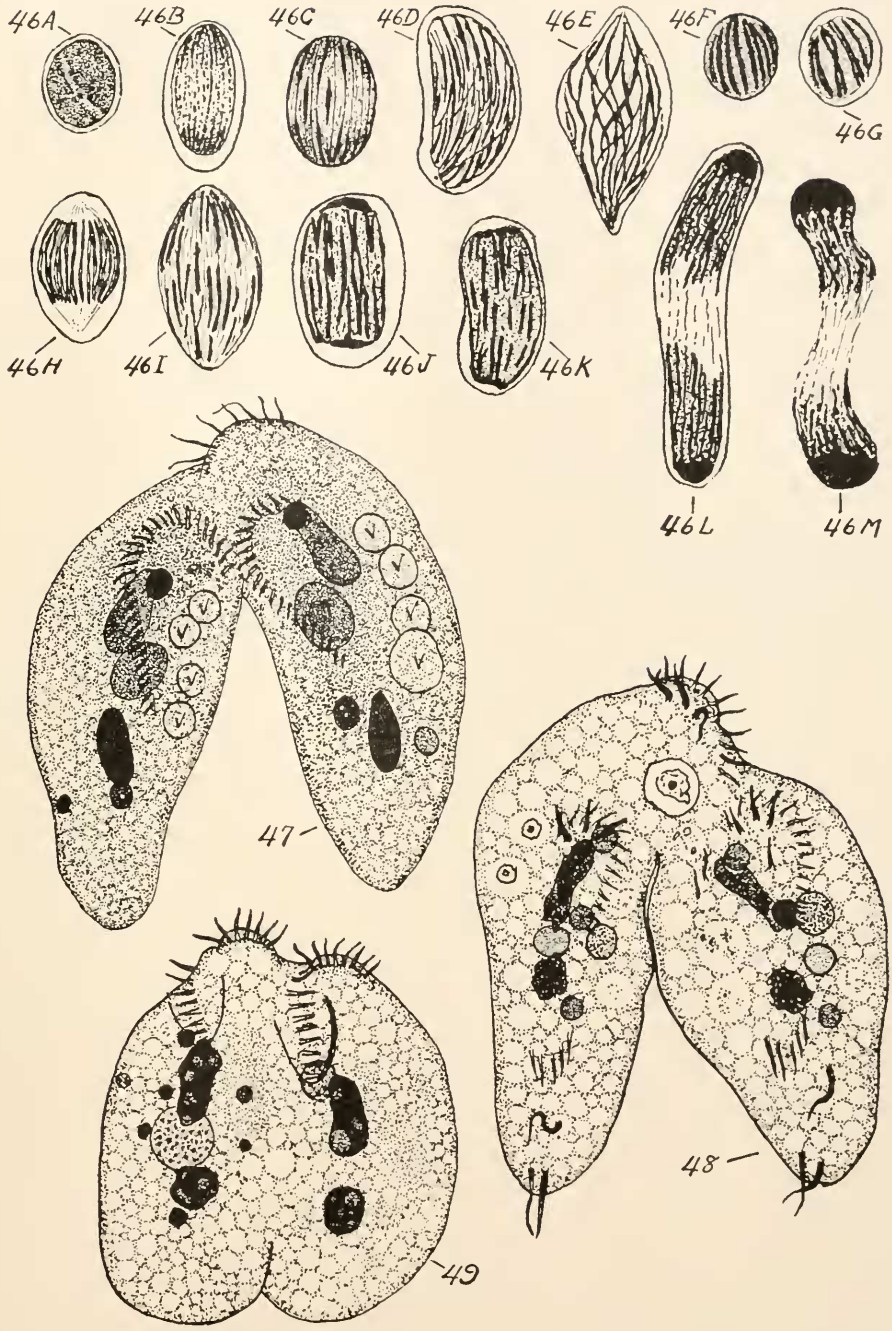

46A
46B
46C
46D
46E
46F
46G
46H
46I
46J
46K
46L
46M
47
48
49

PLATE XII.

Reorganization.

FIG. 50. A pair the members of which are about to separate. The fourth of the nuclei arising from the last cleavage has apparently degenerated in each individual, and the macronuclei have taken on the typical circular and vacuolated appearance which persists until their final degeneration.

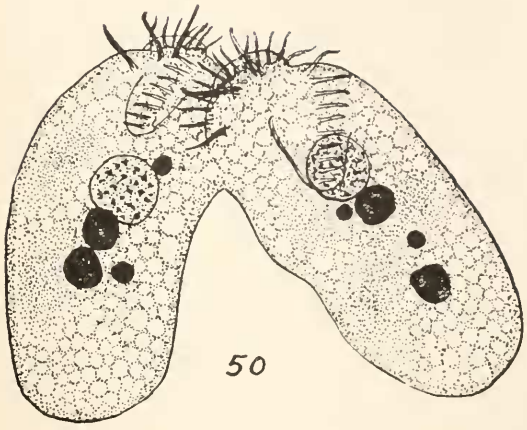
FIG. 51. Exconjugant shortly after separation. Typical of those individuals in which a third cleavage division has occurred.

FIG. 52. Exconjugant somewhat longer after separation than the one shown in Fig. 51.

FIG. 53. Exconjugant in somewhat more advanced stage than the preceding The remnants of the old macronuclei have finally disappeared.

FIG. 54. Reorganization in this individual has apparently been completed without preliminary division.

FIG. 55. One of the daughter individuals produced by the first division of an exconjugant. The macronucleus is in process of division by which the normal nuclear constitution will be finally restored.



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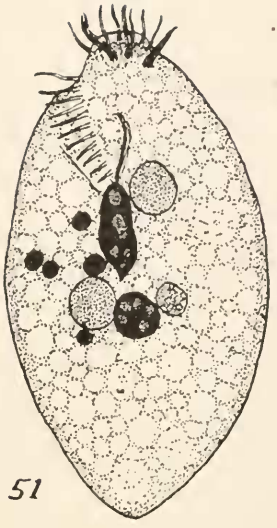

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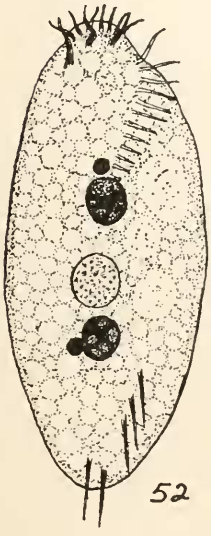

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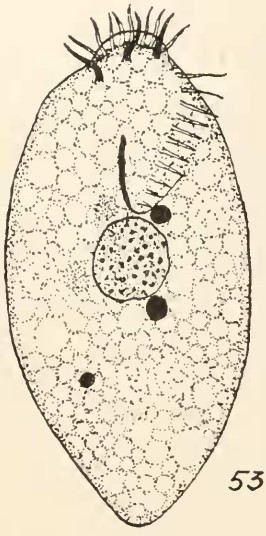

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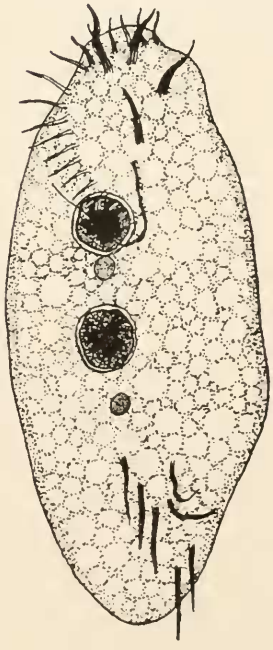

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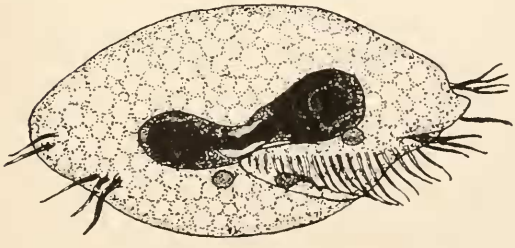

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BIOLOGICAL BULLETIN

NODES AND CHIASMAS IN THE BIVALENTS OF *LILIUM* WITH REGARD TO SEGMENTAL INTERCHANGE.

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The bivalents of several species of *Lilium*, during the interval between the double-thread stage and the metaphase, have already been examined by a number of good observers. Hence for further fruitful work three things seem more or less necessary: á narrowly limited aim; perfect fixation, which is probably best attained by fixing the pollen-mother-cells outside the anther; and accurate microscopy, which will doubtless include the use of a small circular diaphragm on the source of light, a corrected immersion condenser, and appropriate light-filters.

To attain uniform terminology, the writer ventures to use in this paper the terms, diaphase, instead of diakinesis; diplophase, for diplotene stage; etc.

Bulbs of *Lilium longiflorum* (clonal variety, Easter lily) were potted late in the year, in a cool greenhouse. Flower-buds somewhat less than two centimeters long usually showed the required stages of the pollen-mother-cells; namely, late diplophase, diaphase (early, middle and late), and metaphase. One anther at a time could be removed, so that six stages at intervals of an hour or two could be prepared from each bud. The anther was cut up, and the short segments squeezed out in a drop of iron-acetocarmine. (Spreading out the pollen-mother-cells on a slide, fixing with Flemming's solution, and staining with iron-hæmatoxylin or iron-brazilin, gave preparations less suited for the purpose.) The thin layer of liquid was allowed to evaporate under a large coverglass for several hours, until the cells were flattened by the capillary

pressure. Then the edges were sealed (by a thick solution of dammar in xylol, or better) with melted paraffin wax. For oil-immersion objectives the tube-length required to be slightly increased, according to how many microns the chromosomes extended below the coverglass. The apochromatic objective for water immersion, of 1.25 aperture, and the binocular microscope, were mainly used.

Flower buds of *L. regale,* from the greenhouse, gave nearly as clear figures as those of *L. longiflorum.* Buds from *L. candidum,* grown in the open, in June, gave excellent metaphases. But in late July and August, buds of *L. tigrinum, L. speciosum* and *L. auratum* had granular pollen-mother-cells; and though these cleared up in time in acetocarmine, yet they were not so clear at diplophase and diaphase as corresponding stages of *L. longiflorum.* At anaphase also, the chromosomes were shorter and thicker in the plants grown in the summer than in those species raised in the greenhouse in February.

An examination of the chromosomes of *L. longiflorum,* at the anaphase of the first division, shows that there are two large J chromosomes; one with the constriction perhaps one-third from one end, and the other with the constriction near one-quarter from the end. The remaining ten chromosomes have the primary constriction more or less near to, or at, the end; so that the separated segment is small or invisible at this stage. (One of these chromosomes shows a secondary constriction.)

The leptophase and zygophase closely resemble Newton's figures for *Tulipa* (Newton, 1927). In the late diplophase, before the bivalents are separate enough to be countable, nodes may be observed in the double thread. At the earliest diaphase, as soon as separate bivalents can be distinguished, many nodes are visible. It is difficult to find a cell, however, in which all twelve bivalents are free enough from overlap to permit the counting of all the nodes. However, Fig. 1 is a camera drawing of a cell showing the 12 bivalents at the early diaphase. There is here a total of 39 nodes; made up by one bivalent with 5 nodes, four with 4 nodes, four with 3 nodes, and three with 2 nodes. Other cells at the same stage seemed to have about the same numbers of nodes, though all 12 bivalents could not be counted. In *L. regale,* also,

this stage seems to have a larger number of nodes than the following stages; as has, indeed, been noted by previous observers in both plants and animals. In *Tulipa*, which is allied to *Lilium*, Newton's drawings allow the estimation of about 34 nodes in 11 of the 12 bivalents at the latest diplophase or earliest diaphase. Here, there seem to have been one bivalent with 5 nodes, one with 4, seven with 3, and two with 2 nodes.

Between the late diplophase and the late diaphase, many of these nodes disappear. At middle diaphase, so far as incomplete counts have been made, the numbers are less than in early diaphase and more than in late diaphase. At late diaphase, the numbers of nodes in five cells of *Lilium longiflorum*, where all 12 bivalents were widely spaced and the nodes readily counted, were 24, 24, 23, 21, and 21, respectively. The average of these is 22.6. Fig. 2 is a camera drawing of one of these cells. In *Tulipa*, Newton's drawing shows about 21 nodes at late diaphase. Thus in Lilium we have a diminution in the number of nodes, from the earliest diaphase to late diaphase, of 43 per cent.; while in *Tulipa* the estimated loss is also slightly over 40 per cent. It is possible that if the counts could have been made earlier in the diplophase the loss would have been shown to be somewhat larger. Hence we may say that, in *Lilium* and *Tulipa*, at least, somewhat less than half of the original nodes disappear before the metaphase.

At the metaphase, the numbers of nodes were about the same as in late diaphase; namely, 24 in one metaphase of *L. longiflorum* (Fig. 3), and 21 and 20 in two metaphases of *L. regale;* averaging 21.7, as compared with 22.6 for the late diaphase. In these cases the metaphase chromosomes were squeezed from the cells, and cases were readily procured in which the nodes of all 12 bivalents could be accurately counted.

The nodes at late diaphase and at metaphase are doubtless chiasmas (Janssens, 1924), as they are also in *Uvularia*, *Hyacinthus*, and *Allium* (Chodat, 1925). A chiasma can be distinguished microscopically by the following points. (1) When the bivalent is normally flat, or is flattened by pressure, two chromatids may be seen to pass on, and two to form an X. Thus Fig. 4 shows a metaphase bivalent of *L. candidum*, slightly compressed, at three different levels, the focus of the microscope de-

scending from left to right. There are two chiasmas visible, of which the left one is the clearer. Fig. 5 shows another flattened bivalent from the same metaphase plate as Fig. 4, drawn as usual with shifting focus. Here the two chiasmas are clearly alternating with regard to the chromatids. (2) In a chiasma at the diplophase or diaphase, the cross junction can often be seen to be thinner than the continuous threads. In a half turn of a spiral, the cross junction would of course be of double thickness. (3) When sufficient pressure is applied to the diaphase bivalents, after being some days in acetocarmine, they seem to break across only at the nodes. The separation of the strands in a chiasma would doubtless make it a point of weakness. (4) At late diaphase and metaphase, the portions of the bivalents on each side of a chiasma are in planes more or less at right angles to each other (Fig. 3). (5) At the first anaphase, the constituent chromatids do not separate in the halves of vertical V's and rings, while they do in horizontal V's or rings. Fig. 6 shows this separation in *L. longiflorum*.

The nodes (chiasmas) have been counted in 96 bivalents of *Lilium*, from eight different cells, at late diaphase and metaphase. Of these, 31 bivalents had one node, 49 had two nodes, and 16 showed three nodes. Calculating the points of segmental interchange, or points of genetic crossing over, in the resulting chromosomes (four from each bivalent), we find: 119 chromosomes with no point of interchange; 184, with one point of interchange; 73, with two points of interchange; and 8 with three points of interchange. In percentages these are: 31, with none; 48, with one; 19, with two; and 2, with three points of interchange. These figures agree fairly with the figures for the four large bivalents of *Hyacinthus* (Belling, 1927), and perhaps also with the genetical results in species of *Drosophila*.

The nodes which disappear between diplophase and late diaphase do not seem to be all or mainly twists. Clearly recognizable twists (half turns of a spiral) are apparently rare in *Lilium longiflorum* and *L. regale*. Nor do these vanishing nodes seem to be chiasmas which open out; for if so, this process should have been visible, as it is at early anaphase. Their nature awaits further investigation. However, the drawings of early observers, which

show bivalents in *Lilium*, or plants allied to *Lilium*, forming, at the late diplophase or early diaphase, regular right-handed or left-handed spirals of several turns, do not correspond to what is to be observed in *Lilium longiflorum*, with perfect fixation, yellow-green light, a nine-tenths condenser cone, and apochromatic oil-immersion objectives of 1.3 and 1.4 aperture.

Summary.—(1) The bivalents of six species of *Lilium* were studied, from the double-thread stage to the early first anaphase, in pollen-mother-cells fixed in iron-acetocarmine. Thirty-nine nodes were found at late diplophase or early diaphase.

(2) In 5 late diaphases, the average number of nodes was nearly 23, and in 3 metaphases the average number was nearly 22. Thus there was a loss of 43 per cent. of the nodes between late diplophase and late diaphase.

(3) Out of 96 bivalents, there were 31 with one node, 49 with two nodes, and 16 with three nodes. This would result in chromosomes having 31 per cent. with no point of segmental interchange, 48 per cent. with one point, 19 per cent. with two points, and 2 per cent. with three points of segmental interchange.

(4) Dixon's term, "strepsitene," seems to have been a misnomer in the case of *Lilium*. Fifty-seven per cent. of the nodes were demonstrably chiasmas.

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References to earlier literature may be found in these papers.

EXPLANATION OF PLATE I.

FIG. 1. Early diaphase in a pollen-mother-cell of *Lilium longiflorum*. Camera drawing from an iron-acetocarmine preparation (as were also the other five figures). Cytoplasm and chromosomes squeezed from the cell-wall, and flattened on the coverglass. Viewed with the apochromatic oil-immersion objective 60, of 1.3 aperture, used with the binocular attachment. Tube-length increased to correct for watery layer. Wratten film, No. 66, and water-immersion condenser giving aperture of 1.2, used. There were 39 nodes counted. Some of them were seen to be chiasmas, but the detail was too fine to be shown in this drawing. Only one was proved to be a half twist.

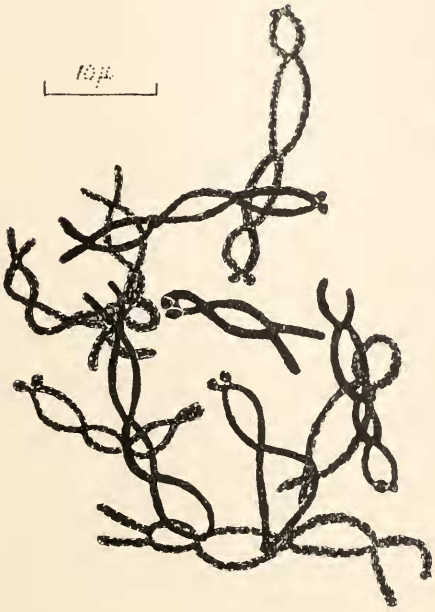
FIG. 2. Late diaphase of *Lilium longiflorum*. Preparation and optical apparatus as in Figure 1, except that lower eyepieces were used. There were 24 nodes. Many could be seen to be chiasmas. Probably all were chiasmas. Stages like this could be had in abundance, and the nodes could be easily counted.

FIG. 3. Metaphase bivalents of *Lilium longiflorum*. Magnification, etc., as in Figure 2. The nodes can be counted with accuracy at this stage, and can be shown to be chiasmas. There were 23 nodes. This stage is common.

FIG. 4. Metaphase bivalent of *Lilium candidum* which has been squeezed flat by due pressure, drawn in three focal planes. Viewed with apochromatic water-immersion objective 70. Description in text.

FIG. 5. Another bivalent from the same cell as that drawn in Fig. 4. Camera drawing with shifting focus of microscope. Described in the text.

FIG. 6. Separation of the homologues in the metaphase and early anaphase of *Lilium longiflorum*. The first four above show stages in the separation of the two larger bivalents. The two below, and the one on the right are shorter bivalents. Chiasmas are evident.



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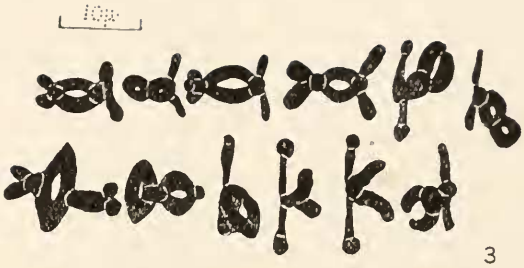

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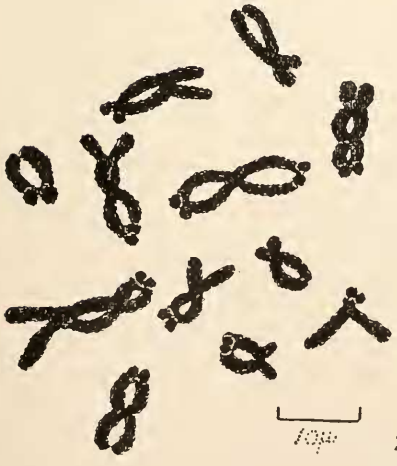
5



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6

JOHN BELLING.

THE NEUROMOTOR APPARATUS OF *CHLAMYDODON* SP.

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Introduction.

While working on the life cycle of *Chlamydodon*, a system of fibers around the mouth was found, and, on account of the relation of these fibers to the pharyngeal basket and other organelles, it seemed worth while to make a further study.

As most of the literature on the subject of conductile fibers, especially in the Protozoa, has been reviewed by Taylor (1920), Rees (1922), and Calkins (1926), it seems unnecessary to take up the subject here.

Kofoid applied the term Neuromotor Apparatus to the complex fibrillar system associated with blepharoplasts, parabasal bodies, etc., found in *Giardia*. Other systems of this kind have been described for the flagellates by other workers.

In the ciliates Sharpe (1915) was the first to give the term Neuromotor Apparatus to a complex system of fibrils, having a center or motorium, which he found in *Diplodinium ecaudatum*. Since that time, similar systems have been described for seven other ciliates as follows: Yocom (1913) in *Euplotes patella;* MacDonald (1922) in *Balantidium coli* and *suis;* Rees (1922), *Paramecium caudatum;* Visscher (1925), *Dileptus gigas;* Campbell (1926 and 1927), *Tintinnopsis nucula* and *Favella;* and Picard (1927) *Boveria teredinidi*. *Euplotes* represents a highly specialized type of Neuromotor Apparatus, *Paramecium caudatum* a generalized type, while that of *Chlamydodon* represents a combination of the two types.

Acknowledgments.

The work was begun at the Marine Biological Laboratory and completed at Agnes Scott College. I wish to thank Dr. Gary N.

Calkins, of Columbia University and the Marine Biological Laboratory, for advice and helpful criticism during the progress of the work, and Miss Ruth B. Howland for help that made the work of microdissection possible.

Material and Methods.

The animals used in this investigation were collected from Knowlton's ditch at Woods Hole, Mass. A small portion of algae, chiefly Oscillatoria, from the same ditch, placed in a syracuse dish in Knop's solution one part, distilled water ten parts, furnished an excellent culture medium. By making sub-cultures, the animals were kept in the laboratory at Agnes Scott College for two winters.

Various killing and fixing solutions were used, the best results being obtained with Schaudinn's Fluid, Bouin and strong Flemming. If the material was to be stained with Mallory's connective tissue stain, it was fixed with Zenker's or Picro-mercuric fluid, using the time schedules recommended by Sharp and Yocom. Some of the best preparations were obtained by the use of .3 per cent. hæmotoxylin, long method.

Two methods of embedding were used: (1) by the aid of a LeFevre embedding dish, and (2), by killing a whole culture in a Syracuse dish, and embedding small bits of algæ to which the animals were attached. This was found to be the most satisfactory method, as there was little danger of losing the animals, and the material could be quickly handled; also it gave an abundance of material. Sections were cut from 2–4μ thick. On account of the thickness of *Chlamydodon,* it is impossible to work out the system of fibrils in whole mounts.

All drawings were made with the camera lucida, except Fig. 15, and with the use of 1/7 Leitz oil immersion lens and 12 $\times$ oculars. Details were worked out with 12 $\times$ oculars and a Zeiss 1.5 mm. apochromatic lens. A 250-watt light in a Zeiss microscope lamp was used for illumination.

The Genus *Chlamidodon.*

The genus *Chlamidodon* was named and described by Ehrenberg in the *Proceedings of the Berlin Academy* in 1835. He states

that he discovered *Chlamidodon mnemosyne* in the waters of the Baltic Sea near Wismar, Aug. 26, 1834. In 1838, in his Infusionsthierchen als volkomene Organismen, he mentions this form again, and, in addition to the "teeth apparatus" mentioned in his first paper, he describes, a "colorless oval shield," projecting on all sides beyond the body, and covering its body. Stein (1859) differs with Ehrenberg in some details, *e.g.*, he says that he found only eight instead of sixteen trichites in the basket, and he thinks the "oval shield" is just part of the body. Entz (1884) describes *Chlamydodon cyclops* Entz as having fifteen and sixteen trichites in the pharyngeal basket, while Erlanger (1890) figures *Chlamydodon mnemosyne* with sixteen. Stein changed the spelling from *Clamidodon* to *Chlamydodon*.

Ehrenberg placed the genus in the family "Euplota"; Stein placed it in the family "Chlamydodonta."

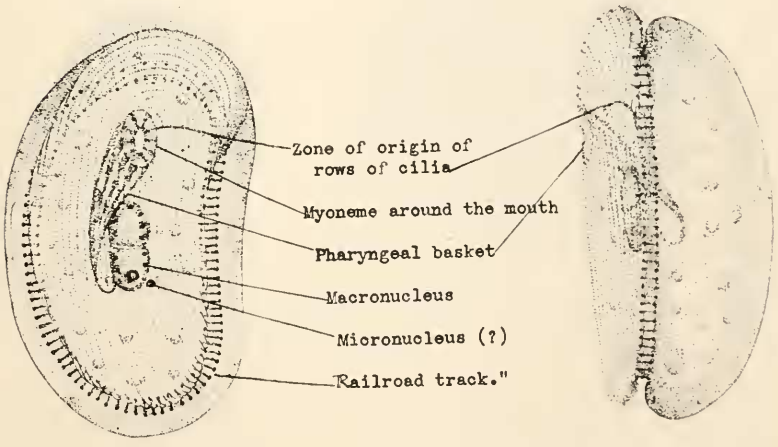


FIG. 1 *A*. *Chlamydodon* sp. (Probably a variation of *mnemosyne*.) Ventral view.

FIG. 1 *B*. Side view.

The form most common at Woods Hole is probably a variation of *Chlamydodon mnemosyne*, though it differs very much from Erlanger's description of that species. There is another species of *Chlamydodon*, found at Woods Hole, which the writer has seen only twice, and only isolated specimens. It has a very short

pharyngeal basket situated at the extreme anterior end. It differs from all described species in the character of its cilia.

The species which was used as a basis for the present study, Fig. 1, measures from 60–70μ in length, and 40–45μ in breadth. Like all of the species, it is ciliated on the ventral side only, the anterior cilia being much longer than those on the other parts of

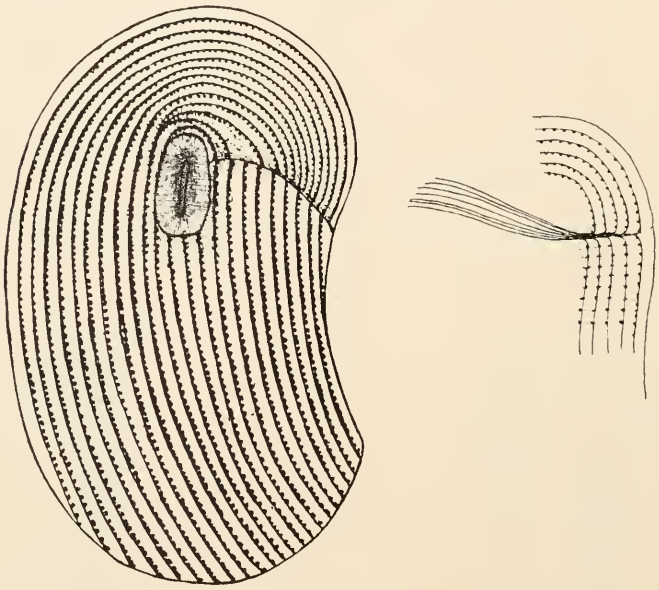


Fig. 2 *A*. Arrangement of cilia and fibers traversing the circular myoneme.
Fig. 2 *B*. Fan of fibers from cilia just before they join motorium. Delicate cross fibers from basal bodies of cilia.

the body. The cilia are very fine, and arranged in rows set close together. To get the true arrangement of cilia, the ventral surface has to be removed and flattened out. This is possible only when the "railroad track" has been cut, as this structure bounds the rows of cilia, and, at the anterior end pulls a part of the ventral surface over on the dorsal side, Fig. 1 *B* and Fig. 4. The anterior cilia take their origin from a zone on the right-hand side (ventral side up), extend around the anterior end in a half circle, thence to the posterior boundary of the "railroad track" in a somewhat curved line, Fig. 2. The cilia below the zone on the right-hand

side, take their origin as said zone, and extend in curved lines to the posterior boundary of the railroad track. The three central rows begin at the posterior end of the myoneme surrounding the mouth, and extend to the posterior end of the " railroad track " in the same manner as the other rows, Fig. 2. Fig. 1 *B* shows the manner in which the " railroad track " bounds the rows of cilia.

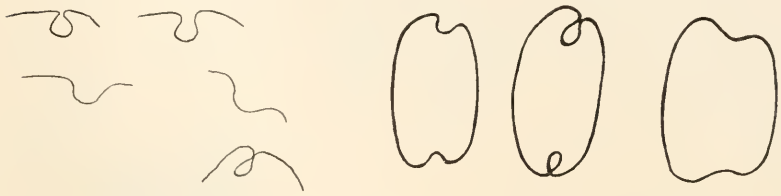


Fig. 3. Sections showing various changes in shape of the " railroad track."
Fig. 4. Sections of entire animal showing changes in the shape of the " railroad track," and changes in the shape of the animal.

The peciliar organelle known as the " railroad track " is a band of trichites encircling the animal in the manner shown in Fig. 1 *B*. In some of the older papers, the exact location of this band is a matter of discussion. We were able to pierce the dorsal wall of the animal with a micro pipette, and blow out the cell contents, including the pharyngeal basket; nothing was left except the body wall, almost entire, and the " railroad track." Its position was unchanged. It is covered with a thin pellicle, and fastened tightly to it. With the micro dissection needles, the track was cut out and pulled apart. While it was easier to tear apart where it was thin, we could not duplicate the effect shown by Erlanger. The figure in Erlanger's paper seems to show the parts of the " railroad track " easily separable into round masses of protoplasm, each having a trichite in the center.

If the animal is viewed from the ventral side, the structure looks flat, and the trichites arranged after the manner of crossties, hence the name " railroad track." A side view of whole mounts, easily obtainable, and sections, shows that the ordinary shape of this structure is half a circle. The organelle has the power of changing its shape. This is readily shown in sections; it may be almost completely closed, or wide open, Figs. 3 and 4. After this

observation was made, a careful study of the living animal showed that when the lip was bent back very far the structure was closed, forming a complete ring. The anterior end was closed during ingestion of food.

The ends of the trichites seem to be heavier than the middle portion, and between each two trichites there is a thin portion with a place in the center easily pulled apart. When the organelle is cut and straightened out, it is seen that the trichite is thickened and the thin portion reduced, the end view reminding one of an accordion, Fig. 17.

FIG. 5. The pharyngeal basket of *Chlamydodon* sp., with circular myoneme which covers it removed.

FIG. 6. Side view, showing relation of basket to myoneme.

The band or "railroad track" is not interrupted, as shown in Erlanger's figures of *Chlamydodon mnemosyne*. Just before division, a break near the center may be observed.

The pharyngeal basket is very large in proportion to the size of the animal. It is very different from the basket described by Erlanger, and figured in Doflein, p. 54. There are ten heavy trichites, the anterior ends being expanded, and the trichites showing distinctly. About half way down, the trichites seem to fuse, their identity becoming lost as the basket narrows, resembling *Chilodon uncinatus* in this respect. The posterior end has a small

filament wound to the right (ventral view). At the anterior end, each trichite has a sort of cap fitted to it somewhat after the manner of a hinge, Figs. 5 and 6, and extending to the mouth opening. These caps are in the shape of a triangle, and form a lid. This lid is, in turn, covered by a circular myoneme, like a sphincter muscle, in the center of which is the mouth opening. As Figs.

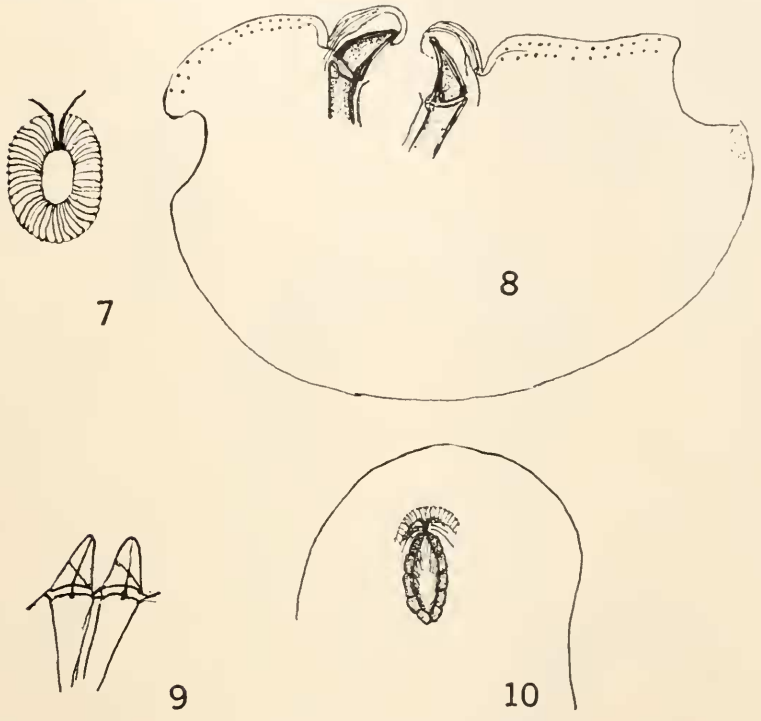
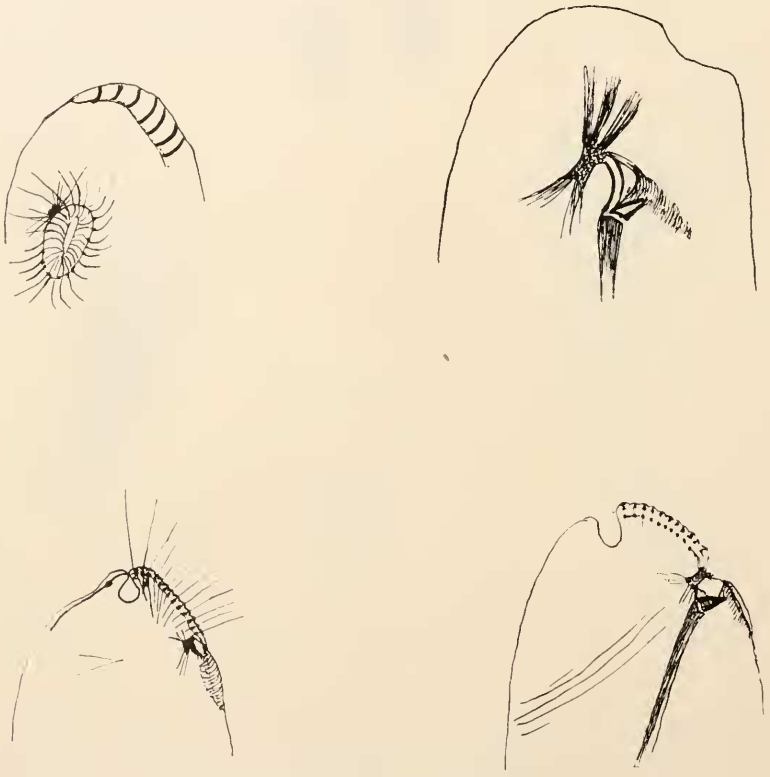


FIG. 7. Circular myoneme surrounding the month, with traversing fibers.
FIG. 8. Tangential section through mouth region, showing relation of basket to myoneme and fibers.
FIG. 9. Fibers in caps of trichites and top of trichites.
FIG. 10. Caps removed from the trichites of the basket. Note fan of fibers.

6 and 8 show, the edges of the mouth opening in this myoneme fit under the points of the triangles. It is suggested that the triangles are pulled back by the myoneme, and so opens the mouth.

The macronucleus is divided into two parts, the anterior part taking the stain more lightly than the posterior half. In the rest-

ing stage, the granules in the anterior half form a complete horse-shoe. These granules change in size and position as cell division proceeds. There are, in addition, many small granules. The posterior half has a large division center, and many other smaller granules. Between the two halves there is a split, or querspalt. Often a granule is found in this split, and it has been observed to divide. The two granules migrate into the cytoplasm. It has not



FIGS. 11, 12, 13, 14. The Motorium, and its relation to other structures.

been possible to follow their further history. In division, the split disappears, the division center moves up the center of the nucleus, pulls out and divides. All of the stages of division have not yet been worked out.

The position of the micronucleus is uncertain. Entz pictures it on the side of the nucleus in *Chlamydodon cyclops,* and Erlanger

shows it imbedded in the anterior end of the macronucleus. It is quite small, and some of my preparations show it at the posterior end of the macronucleus, and some at the side. It cannot be seen in all preparations.

The Neuromotor Apparatus

The first part of the neuromotor apparatus to be observed was a system of fibers around the mouth or oral opening, Figs. 2, 6, 7. As described above, the pharyngeal basket in *Chlamydodon* is very large and heavy, and the circular myoneme around the mouth is seen to be traversed by many fine fibers, with a small granule for

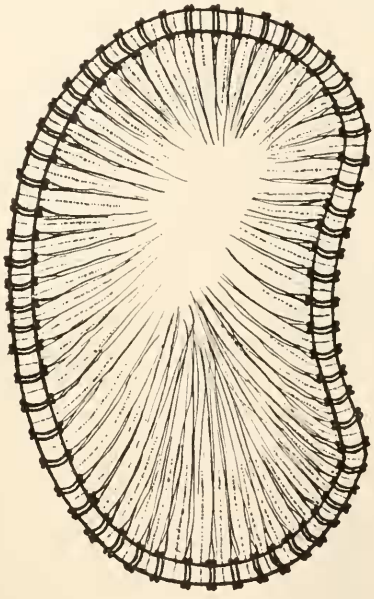


Fig. 15. Diagrammatic representation of the relation of fibers to the "railroad track."

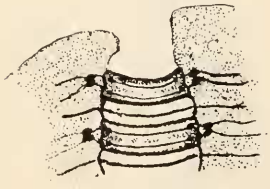
each one on the edge of the myoneme, Fig. 7. In only one or two favorable preparations could it be seen that these fibers run out into the cytoplasm in the general direction of the "railroad track," Fig. 11. With the aid of micro dissection needles, it is possible to lift off the ventral surface of the animal. If this is

then properly stained, it is seen that the myoneme around the mouth is continuous with the body wall, but much thicker. The fibers from the myoneme extend under the rows of cilia. They are very fine, and it is impossible to trace them far.

Fibers run from one trichite to the next, and there are cross fibers on each of the caps, Fig. 9. These fibers all come together in two heavy strands, joining other fibers around the mouth, and connecting the motorium at the lower right-hand corner, Figs. 12 and 13.

The basket seems to be lined with a very thin membrane, and when the caps are removed from the trichites, fibers can be seen in this lining, Fig. 10.

The motorium is just below the anterior end of the basket, and is placed a little slantingly, Figs. 11, 12, and 13. It is a bilobed mass, which stains with hæmotoxylin. It, and the other fibers in the system, stain bright red with Mallory's connective tissue stain.



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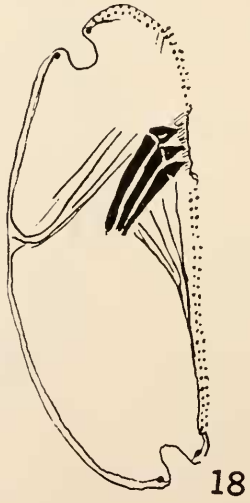

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Fig. 16. Portion of the "railroad track" greatly enlarged.

Fig. 17. Portion of the "railroad track" dissected out and flattened. Shows peripheral fibers.

Fig. 18. Section showing dorsal and ventral fibers from the "railroad track."

It is impossible to see the motorium in the whole mounts. On account of its proximity to the pharyngeal basket, it was overlooked for a long time, for the basket is heavy, and when destaining is carried on long enough to differentiate it, the motorium is destained. In sections, however, the structure is easy to see. With the aid of microdissection needles, the basket can be dragged out whole, and the motorium is sometimes pulled out with it. It is then seen to be a refringent body, while in stained sections its general structure appears granular. Fig. 12 shows the fans of fibers joining the motorium.

The peculiar organelle known sometimes as the "striped band" or "railroad track" mentioned above, has a very complex system of fibers connected with it. Sections show the fibers very well, but their paths are hard to trace on the ventral side on account of the presence of the cilia.

At each end of each trichite, there is a plate or mass, Fig. 16. In sections this mass appears single, Figs. 13 and 18; when viewed from the ventral side of the animal, it appears bilobed or double, Fig. 16. There is a possibility that when the granules were observed from the ventral surface, they were in the process of division, but, when they were seen at all from this angle, they appeared double. The presence of fine rows of cilia on the ventral surface obscures everything; on the dorsal surface the body is much curved in the region of the railroad track, making it impossible to see the granules or masses so close to the organelle. The fibers that go through the trichites are connected with these masses. Fig. 15, in a somewhat diagramatic way, shows the relation of one set of ventral fibers with the masses and the trichites. There are two sets of fibers, one set that enters the trichites, and one set runs through the thinner portion of the "railroad track," Fig. 15. These fibers are from two levels. When the mouth is open, during the ingestion of food, the railroad track changes its shape from a half circle to a closed circle, Fig. 4. From the evidence of the sections, and the observations upon the living material, it is reasonable to suppose that one set of these fibers is connected with the myoneme around the mouth. The presence of ciliary lines and fibers make it almost impossible to trace these fine lines.

The dorsal fibers extend only a short distance underneath the pellicle, then they turn and go in the direction of the motorium.

Only two preparations showed with any clearness the fans of fibers joining the motorium. The basal bodies of the cilia are connected both by longitudinal and cross fibers, Fig. 2 *B*. The longitudinal fibers of the cilia turn in at the zone of origin of the cilia, and connect with the motorium at the anterior end, Fig. 12. This end of the motorium also receives the fibers from the ventral surface of posterior end of the animal. Fig. 15, and some fibers from the mouth region. Figs. 13 and 14. The dorsal fibers join the motorium at the posterior end.

As described above, between each two trichites, there is a thinner portion, and in the center of this is a series of small granules. The granules are connected with fibers which pass over the trichites to the next set of granules, Fig. 17. In some sections, the accordion like arrangement is more pronounced than others, suggesting that there is power of movement, a sort of folding of the trichites. No observations on the living material settled this point, but further evidence of the possibility of the movement suggested lies in the fact that the trichites are sometimes closer together than at other times.

Microdissection.

After the location of the motorium, twenty-five animals were successfully cut, freehand, with the aid of micro-dissection needles given to me by Dr. Robt. W. Chambers of Cornell Medical College. Later, Miss Howland, of New York University, operated upon several with the Chambers micro-dissection apparatus.

The cilia of *Chlamydodon* are fine, the anterior ones being longer and easier to observe than the posterior ones. The motorium cannot be seen in unstained specimens but its position with relation to the basket is known, so that it is quite simple to destroy it. If the motorium be destroyed, there is a marked disturbance in the action of the cilia, in no way comparable to the disturbance of the cilia if other parts of the body are injured. The cilia still have their wave like motion, however, and this is to be expected when one takes into consideration the relation of connecting fibers of the cilia, Fig. 2 *A* and *B*. However, the cilia do not reverse after

the destruction of the motorium as is usual in intact animals. Isolated pieces behave the same way, as has been observed by Jennings and Jamieson (1902), and by Rees (1922). Animals without conspicuous motile organs are not favorable material for the study of the coördination by means of microdissection.

Summary.

A description of *Chlamydodon*, probably a variation of the species mnemosyne, found in brackish water at Woods Hole, is presented.

There is a complex neuromotor apparatus, including a coördinating center, and systems of fibers connected with cilia, the mouth opening, the phryngeal basket, and the "railroad track."

The connection of the fibers with the organelles and the central mass, or motorium, the behavior of the animal after the destruction of the motorium, seem to suggest a coördinating function for the system.

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OBSERVATIONS ON THE FOOD REACTIONS OF *ACTINOPHRYS SOL*.

JAMES BURDINE LOOPER.[1]

Introduction.

Observations on the ability of the protozoa to choose their food date back to 1838 at least. At that time Ehrenberg observed that various organisms ingest carmine which, as food, is nothing more than inert matter. From 1838 until 1902 most of the workers in this field held that protozoa do not possess the power of choosing their food. Even at the present time, as stated by Visscher ('23) in the introductory to his work on *Dileptus gigas,* it is evident that the selection of food has thus far been positively demonstrated in a very few forms.

Kepner and Whitlock ('21), in their work on *Amœba,* give proof of qualitative efforts on the part of *Amœba* to meet certain contingencies. Up to the present time proof of such qualitative effort in other rhizopods has not been recorded so far as I have been able to determine.

This work was done under the direction of Professor William A. Kepner, to whom I am deeply indebted for helpful suggestions regarding these experiments and the preparation of this paper. I am also indebted to Professor Bruce D. Reynolds for valuable criticisms and suggestions.

Culture Method.

The best cultures were obtained in an infusion made by boiling three grains of wheat for five minutes in 100 cc. of filtered spring water. The infusion, along with the three grains of boiled wheat, was placed in a small, clean, glass aquarium and allowed to stand at least twenty-four hours before the animal was introduced. After about a month had passed *Actinophrys* could be secured

[1] From the Laboratory of Biology of the University of Virginia, and the Laboratory of Histology, University of Mississippi.

from such a culture usually in abundance. The cultures were kept covered with thin sheets of plate glass and were kept in the darkest portion of a well lighted room. To keep the cultures going it was found necessary to add to each culture 5 to 10 cc. of boiled spring water per week. The Heliozoa subsisted chiefly upon *Peranema* and *Colpidia*, the progenitors of which were introduced when the cultures were inoculated.

Methods of Observations.

All observations were made under the compound microscope —the 4 mm. objective and number 10 eyepiece being used in practically every observation made. A number 10 eyepiece containing a corrected micrometer, and having a camera lucida attached was kept at hand in case sketches or measurements were to be made.

Cultures in Petri dishes were examined under the 16 mm. objective. In this way observations were obtained on movement and general behavior in a habitat to which the animals were thoroughly accustomed.

A few observations were made on specimens in a drop of water on an ordinary micro-slide under a cover glass, but most observations were made on specimens mounted in a hanging drop. The former method is inconvenient because water has to be supplied to compensate for evaporation. This often disturbed the *Actinophrys* in such a way as to cause it to lie quietly for some time, hence, much valuable time may be lost. The hanging drop method alleviated this trouble once the mount was properly made. To prepare the mounts, first a glass ring about 1 cm. in height and 1½ cm. in diameter, was placed on a clean slide and attached thereon by applying vaseline around the proximal end of the ring. The ring was then supplied with well-oxygenated water until the meniscus came up to half the height of the ring. Clear water in which *Elodea* or *Chara* was actively growing was found to be best. The supply of oxygen in the water in the ring tends to replenish the supply in the hanging drop as it is used up by the mounted *Actinophrys sols*. The desired number of the above mentioned specimens was then isolated by means of a capillary pipette and placed on a clean number one coverglass. Next, with another

capillary pipette, the desired objects of prey were applied to the drop on the coverglass containing *Actinophrys sol.* The upper end of the ring, which had previously been prepared, was smeared with a small amount of vaseline. The coverglass was then inverted and gently pressed down on the ring. Mounts, prepared as described above, often lasted two weeks for observation. In observing these mounts under the 4 mm. objective, better lighting was obtained by removing the sub-stage condenser and employing the concave mirror.

Special Pseudopods.

The special pseudopods for taking in prey are always composed of hyaline ectoplasm. There are three general types of these special pseudopods. First, if the object to be ingested is very small and relatively motionless, a small, straight pseudopod is extended, and upon coming in contact with the object spreads out into a cup which encircles and closes in closely on the object. These pseudopods often resemble a dipper if the protruding end happens to pass beyond the food object before contact is made. Second, if the motionless object be large, a wide, hyaline outgrowth (Fig. 1, 1 *a*) of protoplasm advances towards the prey. When it gets quite near to, or comes in contact with the prey, the tip of this special pseudopod expands in all directions laterally. These lateral expansions close about the large motionless food object usually in close contact with it. In the few instances when close contact may not be made about the large motionless food object, the food vacuole is always decidedly smaller than is the case with animate objects mentioned in the next or third class. Third, large sack-like pseudopods (Fig. 2, 1 *a*) are sent out to encircle active animals. The victim is often cut off from a means of escape by a palisade of rays on one side, and by the approaching special pseudopod on the other.

Reactions to Inanimate Objects.

In experiments on reaction to inanimate objects, only such materials were used that were thought to be non-toxic to *Actinophrys.* In addition, the materials chosen were of such nature as to be easily distinguishable in the vacuoles. The following were tried:

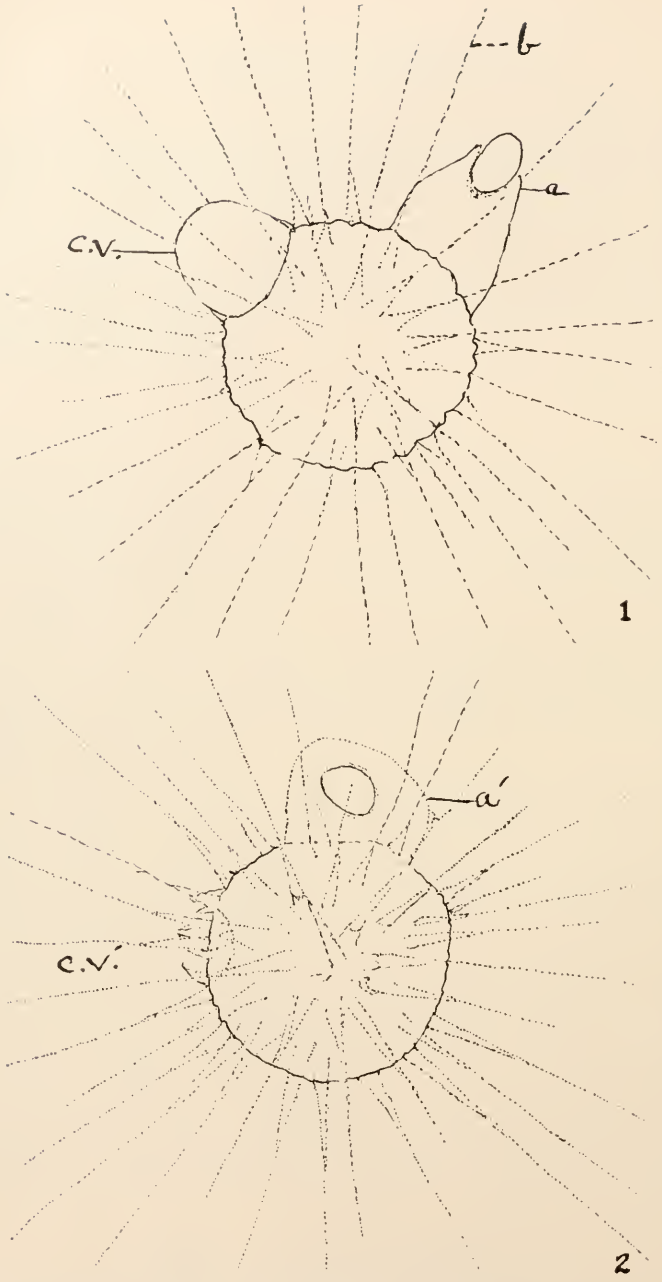

FIG. 1. Sketches illustrating the ingestion of an inanimate object—granule of wheat starch.

1. *Actinophrys sol* with the granule partially enclosed by the food-getting pseudopod, *a*; *b*, ray; *c.v.*, contractile vacuole.

2. Same specimen one half hour later in which *a* has grown to *a'*. *c.v.'*, Protoplasmic projections representing broken wall of emptied contractile vacuole. (× 825.)

carmine, alizarine, powdered glass, plain wheat flour, corn starch, and powdered graphite. Ten experiments were tried with each of these materials, but evidence of ingestion was seen only in the experiments where carmine, wheat flour, and corn starch were used. In the experiments with each material care was taken to try individuals which were in different physiological conditions; *e.g.*, small, underfed specimens, well fed specimens, and specimens which were accustomed to different types of food. Only experiments with materials which *Actinophrys* ingested will be recorded in this paper. The following tables contain the results:

TABLE I.

REACTIONS TO CARMINE.

Experiment.	No. of Individuals in Hanging Drop.	No. which Did Not Take Any Particles within 2 Hrs.	No. Taking Only One Particle within 2 Hrs.	No. Taking Two Particles within 2 Hrs.	No. Taking Three Particles within 2 Hrs.	No. Taking More than Three Particles within 2 Hrs.
1.....	8	5	2	1	0	0
2.....	5	4	1	0	0	0
3.....	11	9	1	1	0	0
4.....	7	5	1	0	1	0
5.....	4	3	1	0	0	0
6.....	5	3	2	0	0	0
7.....	6	5	0	1	0	0
8.....	3	2	1	0	0	0
9.....	15	12	2	1	0	0
10.....	5	4	1	0	0	0
Total.....	69	52	12	4	1	0
Per cent.....	—	75.4	17.4	5.8	1.4	0

In each experiment observations were made as soon as the hanging drop mount was made, then every fifteen minutes thereafter for a period of two hours. In no case did it seem probable that ingestion followed by egestion took place within the time between observations. This is because *Actinophrys* reacts slowly.

Table I. shows that fifty-two individuals (75.4 per cent.) did not take carmine at all. Twelve individuals (17.4 per cent.) took only one particle of carmine. The number taking two particles was four, only one individual took as many as three particles, while none took more than three. Hence, it is evident that car-

mine was ingested by only about one fifth of the total number of individuals, and these took carmine in relatively small quantities.

I was fortunate in seeing eight of the specimens ingest the particles. In each case small, straight, food-getting pseudopods were extended and the particles were closely embraced. This phenomenon will be referred to again later.

Table II.

Reactions to Wheat Flour.

Experiment.	No. of Individuals in Hanging Drop.	No. which Did Not Take Any Particles within 2 Hrs.	No. Taking Only One Particle within 2 Hrs.	No. Taking Two Particles within 2 Hrs.	No. Taking Three Particles within 2 Hrs.	No. Taking More than Three Particles within 2 Hrs.
1.....	5	2	1	2	0	0
2.....	5	3	2	0	0	0
3.....	7	4	2	0	1	0
4.....	4	2	1	0	0	1
5.....	9	5	3	1	0	1
6.....	7	6	0	1	0	0
7.....	10	7	2	0	1	0
8.....	3	2	1	0	0	0
9.....	6	3	2	1	0	0
10.....	8	5	2	1	0	0
Total.....	64	39	16	6	2	2
Per cent.....	—	61	25	9.3	2.3	2.3

In Table II. are found the results of ten experiments with plain wheat flour. Thirty-nine individuals (61 per cent.) did not ingest particles of the flour. Twenty-five per cent. ingested only one granule each, and three tenths per cent. ingested two granules, while two and three tenths per cent. ingested three and four granules, respectively. None were observed to take in more than four granules.

The experiments with flour were carried out under practically the same conditions as the experiments with carmine. However, the results of the former tend to show that particles of wheat flour are taken in by a larger number of individuals, and in relatively larger amounts, than carmine was by individuals under similar conditions. This can probably be explained by the fact that particles of wheat flour may be coated with a thin film of protein material which *Actinophrys* is able to digest and assimilate,

though I was not able to detect any change in granules which lay in the food vacuoles for as long as five days.

The type of pseudopod employed in engulfing particles of wheat flour belongs to the second class previously described for the taking in of relatively large motionless objects. As with the carmine particles, the pseudopod closely embraces the particles of flour, though the process is very much slower. The slowness of ingestion is probably due to the larger size of the particles.

Table III. contains the results of ten experiments with corn starch. Fifty-eight individuals (85.3 per cent.) did not take in granules at all. Eleven and seven tenths per cent. engulfed only one particle each. Three per cent. took in two particles each,

TABLE III.

REACTIONS TO CORN STARCH.

Experiment.	No. of Individuals in Hanging Drop.	No. which Did Not Take Any Particles within 2 Hrs.	No. Taking Only One Particle within 2 Hrs.	No. Taking Two Particles within 2 Hrs.	No. Taking Three Particles within 2 Hrs.	No. Taking More than Three Particles within 2 Hrs.
1.....	12	10	2	0	0	0
2.....	8	8	0	0	0	0
3.....	5	4	0	1	0	0
4.....	9	7	1	1	0	0
5.....	9	8	1	0	0	0
6.....	3	3	0	0	0	0
7.....	6	5	1	0	0	0
8.....	5	3	2	0	0	0
9.....	5	4	1	0	0	0
10.....	6	6	0	0	0	0
Total.....	68	58	8	2	0	0
Per cent.....	—	85.3	11.7	3	0	0

while none took in more than that number. By referring to Tables I., II., and III., it may be easily seen that these results show that corn starch was taken in by fewer individuals than either carmine or wheat flour.

The method of ingesting corn starch was observed to be practically identical with the method referred to under experiments with wheat flour, viz., inanimate particles are closely embraced by the food-getting pseudopods.

Since it is more convenient to merely state the results of the

following experiments, tables will not be given. In all experiments the procedure as outlined under the reactions to inanimate objects was followed and the data were obtained in a corresponding manner.

Reactions to Motionless Animate Objects.

Under this heading reactions to living yeast, motionless algæ, and *Euglena* cysts are recorded.

A. Desmids.

a. Scenedesmus.—In experiments with *Scenedesmus* forty-eight *Actinophrys* were involved. Sixty per cent. did not take the algæ. Those engulfing only one mass constitute thirty and three tenths per cent. The remaining nine and six tenths per cent. of the individuals engulfed two masses each. These desmids were taken in by the same type of pseudopod as were the inanimate objects.

b. A Larger Desmid.—Fifty animals involved in ten experiments were tried with this desmid. Only four per cent. attempted to engulf the larger algæ, and in one case only was the attempt successfull. In this case one *Actinophrys* sent out a pseudopod and partially surrounded the plant but was not large enough to completely surround it. This animal, while attempting to surround the desmid, was joined by another animal. This union appeared to be a complete fusion of the cytoplasm of one with that of the other, the nuclei remaining separate. After this fusion the pseudopod of the first animal was enlarged to completely surround the desmid. The desmid remained in the food vacuole of the multiple individual for a period of five hours and was then slowly forced out through the outer membrane. Nothing remained of the plant except the cellulose wall and a few particles which remained on the inside of it. A similar phenomenon in the Rhizopods was referred to by Delage and Herouard ('96), as a "*Societe de Consumation.*" Distaso ('08) called attention to the grouping of *A sol* for the capture of large food objects.

B. Yeast.

In experiments with yeast seventy-two animals were used. Twenty per cent. of these took in one or more of the plants.

Since yeast plants are so small it was impossible to keep check on the number taken in. When taking in objects of the above kind the pseudopod was of the small straight type referred to previously in this paper. The plants in all cases observed were closely embraced by the pseudopod until digestive fluid began to collect around them, filling a space between the outside of the plants and the wall of the vacuole.

C. Cysts of Algæ.

Euglena cysts and cysts of *Chlamydomonas* were taken in in practically the same proportion. In each case one cyst each was taken in by fifteen individuals (20 per cent.). Four per cent. took in two cysts each, while only one per cent. ingested three each. There were not any taking in more than three cysts. The close embrace was employed in all ingestions observed.

The experiments with motionless animate objects show that *Scenedesmus* is taken more often than either living yeast, *Euglena* cysts, or cysts of *Chlamydomonas;* also, that a larger desmid was rarely chosen as an object of food. The ingestion of the larger algæ required the coöperation of two or more individuals.

Reactions to Moving Animate Objects.

In reacting to moving animate objects *Actinophrys* presents an entirely different type of food-getting pseudopod. Previously, this was referred to as a relatively large, cup-like pseudopod. Instead of the close embrace of the object by the pseudopod, which was referred to under reactions to inanimate objects, we have what may be referred to as a "subtle embrace." A description of the capture and ingestion of a *Colpidium* will serve to make clear what is meant by a "subtle embrace."

While watching the movements of an *Actinophrys* a *Colpidium* was observed to encounter the tips of some of the rays of the former. The *Colpidium* became motionless almost instantly, as if completely paralyzed by the encountered rays. While watching to see the ultimate fate of the ciliate a relatively large mass of cytoplasm was observed to be protruding from the surface of the *Actinophrys*. This mass, which proved to be a food-getting pseudopod, came forward as a single mass until it was four micra

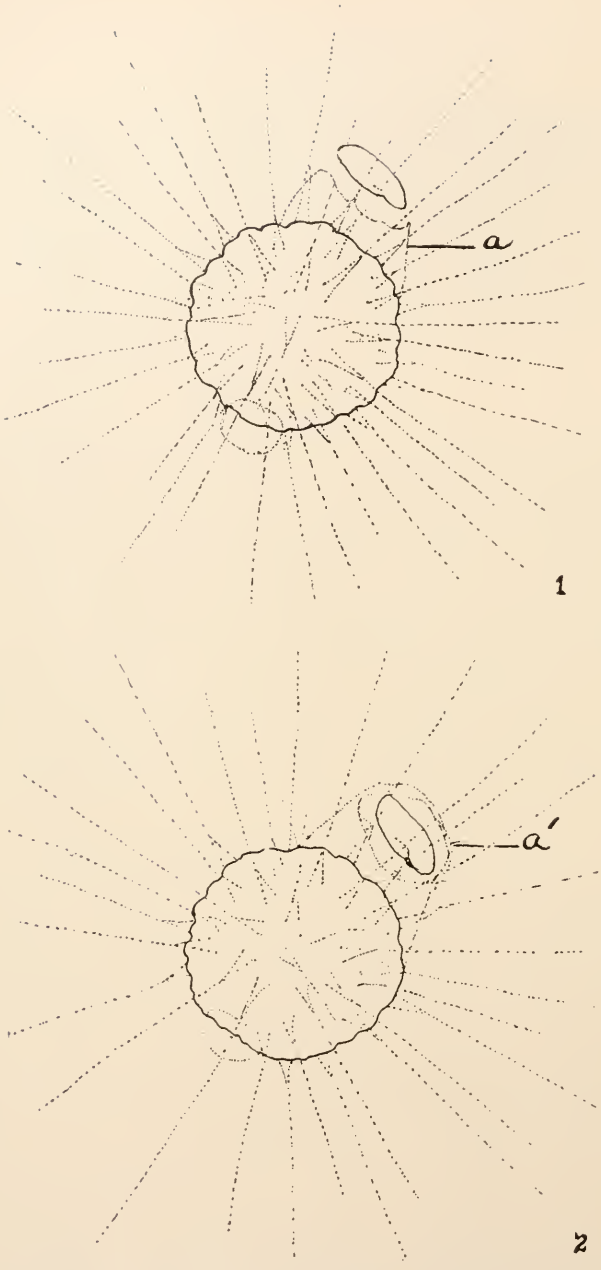

FIG. 2. Sketches illustrating the ingestion of a moving animate object—*Chilomonas*.

1. *Actinophrys sol* with a pseudopod, the contour of which is becoming adapted to that of the prey before coming into contact with the latter. *a*, food-getting pseudopod.

2. Same specimen fifteen minutes later. *a* has grown to become *a′*, which shows wide detour about the motile prey. (×750.)

away from the quiet *Colpidium;* then, without approaching farther, it gave off two branches (Fig. 2, 1 *a*). These branches continued to protrude, encircling the victim, and fusing on the opposite side of it. Thus far, the *Colpidium* was not approached closer than two micra by the branches of the engulfing pseudopod. Hence, the pseudopods were thrown subtly, but not intimately, around the prey. Immediately after the fusion of the pseudopods beyond the prey they spread in such a way as to form a hollow sphere or vacuole containing the *Colpidium* on the inside (Fig. 2, 2). The vacuole then contracted around the prey, which in the meantime became stimulated to exhibit frantic efforts, apparently for the purpose of escape. These efforts were kept up for a period of at least five minutes before the imprisoned animal finally became quiet again. The vacuole containing the prey was eventually incorporated into the body and grew smaller as digestion proceeded, and disappeared completely when the indigestible particles were finally ejected through the cell membrane of *Actinophrys.*

A. Ciliates.

Colpidium.—Ten experiments with *Colpidia* reveal the fact that forty individuals (50 per cent) took in one or more *Colpidia.* Thirty-seven and seven tenths per cent. engulfed one *Colpidium* each. Twelve per cent. took in two specimens each; while only three tenths per cent. engulfed three each. Apparently, the reason why there was such a small per cent. taking in as high as three each lies in the fact that *Colpidium* is relatively too large to be engulfed in larger numbers by *Actinophrys* within two hours.

Loxocephalus granulosus.—*Loxocephalus* is taken in very readily and in relatively large numbers by *Actinophrys.* In the experiments with the above mentioned ciliate only thirty-six (40 per cent) of the Heliozoans failed to ingest one or more. Thirty per cent. of the individuals engulfed one ciliate each. Two each were taken in by twenty per cent., while ten per cent. were observed to have three vacuoles containing one *Loxocephalus* each. The type of pseudopod used in taking in this organism was observed to be practically identical with the type described under reactions to *Colpidium.*

Stylonychia mytilus.—This ciliate is much larger and stronger

than *Actinophrys*. Some of the cilia have been modified to form bristle-like cirri which may be used as organs of offence, or defence. Hence, it is hardly possible for *Actinophrys* to capture such an organism single-handed, and so far as known no one has ever observed such. I have seen four different cases where individual *Stylonychia* were engulfed by a group of *Actinophrys*. These groups in two cases were assembled after one *Actinophrys sol* had tried to capture and ingest the larger and stronger animal. In the other two cases the groups had been previously formed. In no case was a *Stylonychia* taken in by a group of less than four individuals. The group action here, if it may be called group action, differs in at least one respect from the association of individuals described by Kepner ('25). In the case he reported, three individuals united to take in a motionless desmid, and the pseudopods of the former embraced the latter closely. In the case of a group taking in a *Stylonychia* the pseudopods presented the phenomenon of the loose, or subtle embrace described under reactions to *Colpidium*.

Other Ciliates.—Ten experiments were carried out with *Frontonia lucas*, *Nassula ornata*, and *Spirostomum ambiguum* respectively. Some of the experiments in each case were kept under observation for five days. However, in no case were any of the above-mentioned ciliates engulfed by *Actinophrys;* while *Colpidia* and *Chilomonads,* which happened to be among these ciliates, were all engulfed sooner or later. This certainly indicated a definite discrimination in regard to the type of ciliate taken in. On what is this discrimination based? Whatever the basis, it is hard to believe that either *Frontonia, Nassula* or *Spyrostomum* would be more difficult, from a mere physical standpoint, to capture and engulf than *Stylonychia*.

B. Reactions to Flagellates.

Chilomonas paramecium.—Reactions to this flagellate were practically the same as described under reaction to the ciliate, *Colpidium*. Forty-one individuals (51 per cent.) of *Actinophrys sol* did not engulf *Chilomonas*. Of the remaining forty-nine per cent. forty per cent. engulfed one each. Six per cent. took in four each.

Peranema trichophorum.—*Peranema* proved to be engulfed

very readily by *Actinophrys*. Only twenty-five individuals (42 per cent.) failed to take in this flagellate. Thirty-five per cent. engulfed one per individual. Nineteen and one tenth per cent. took in two each, and three per cent. three each. In no case were as many as four *Peranema* engulfed by an individual. The complete process of capture and ingestion was observed in eleven cases. In each the subtle embrace was employed.

Euglena viridis.—Euglena viridis was taken more readily than any other food material. Thirty-five individuals (58.4 per cent.) of the Heliozoan ingested one each. Ten per cent. took in two each, and one per cent. three each.

Euglena, as a rule, was embraced more closely than either of the other moving animate objects. I have not been able to observe definitely why this was the case. However, this deviation from the usual procedure in ingestion of moving animate objects probably has some connection with the fact that *Euglena* was often brought in contact through a reaction with the body proper of *Actinophrys* before a food-getting pseudopod was sent out. This contact with the body proper was brought about by a sudden jump of *Euglena* in the direction of the body proper of *Actinophrys* while reacting to a contact with the rays. The exact reason for this sudden jump on the part of *Euglena* has not been determined, though it has been suggested that the long flagellum of *Euglena* became attached to the body of *Actinophrys*, probably, by a mucilaginous property of the latter. Then in an effort to free itself, the *Euglena* pulled itself into contact with the body of *Actinophrys* to which the end of its flagella was fixed. Thus the body of the flagellate became fastened by the same property. If this be true, the food-getting pseudopod which arises from the outer region of the Heliozoan's body, would quite probably adhere closely to the flagellate's body as it encircled it. Should, however, the *Euglena* begin to squirm, the cup of the pseudopod would open up to form a larger cavity around the victim. Thus in case the *Euglena* began to struggle, what was started as a close embrace was forthwith transformed into a subtle embrace.

D. Reactions to Rotifers.

Only individuals belonging to one or two of the small species of Rotifers were taken in by *Actinophrys*. Rotifers are the only

multicellular animals which were observed to be captured and ingested by the Heliozoan. Here, as with *Stylonichia,* a group of less than four was not strong enough to capture a Rotifer. I was fortunate to see the complete struggle involved in the capture and ingestion of five specimens by five different groups of *Actinophrys.* Here, as in the case of the capture and taking in of *Stylonychia,* some of the groups were formed after a single individual had become attached to the prey (Fig. 3). In the formation of two of these groups individuals which were entirely out of the low power field of the microscope joined the fray, fusing with their comrades which were already involved. In one of the above mentioned cases four individuals were not in the field when the Rotifer was encountered by an *Actinophrys* which was in the field. In the other case, likewise, three were outside of the field of vision. In these cases the rays of the individuals which came into the field of the microscope to join in the feast were not adjacent with the rays of those already involved. This seemed to indicate that there must be some way of sending out a call for assistance when the object was too large and pugnacious to be conquered and swallowed by one individual. The reason that it cannot be said definitely that this is the case is because the outsiders might be drawn in by currents set up in the medium by the floundering of the attacked prey. The fact that a number of *Actinophrys sol* fused to form an aggregate when brought together by agitating the surrounding medium with a glass filament, strengthens the above mentioned idea. Whatever may be the cause of the formation of temporary colonies, the phenomenon is, at least, of interest. By virtue of this fact, it becomes easier to conceive of how multicellular animals may have arisen by the association and coöperation of individual cells. Be this as it may, the formation of multiple individuals by *Actinophrys sol* makes possible the capture and ingestion of larger objects of prey. This is essentially in agreement with Distaso's ('08) observations on *A. sol.* Apparently this temporary colony formation also serves a protective rôle. It is probably of interest to note that an animal so low in the scale of organic life exhibits the phenomenon of coöperation in the fundamental activities of its life.

By comparing the results of the experiments carried out with

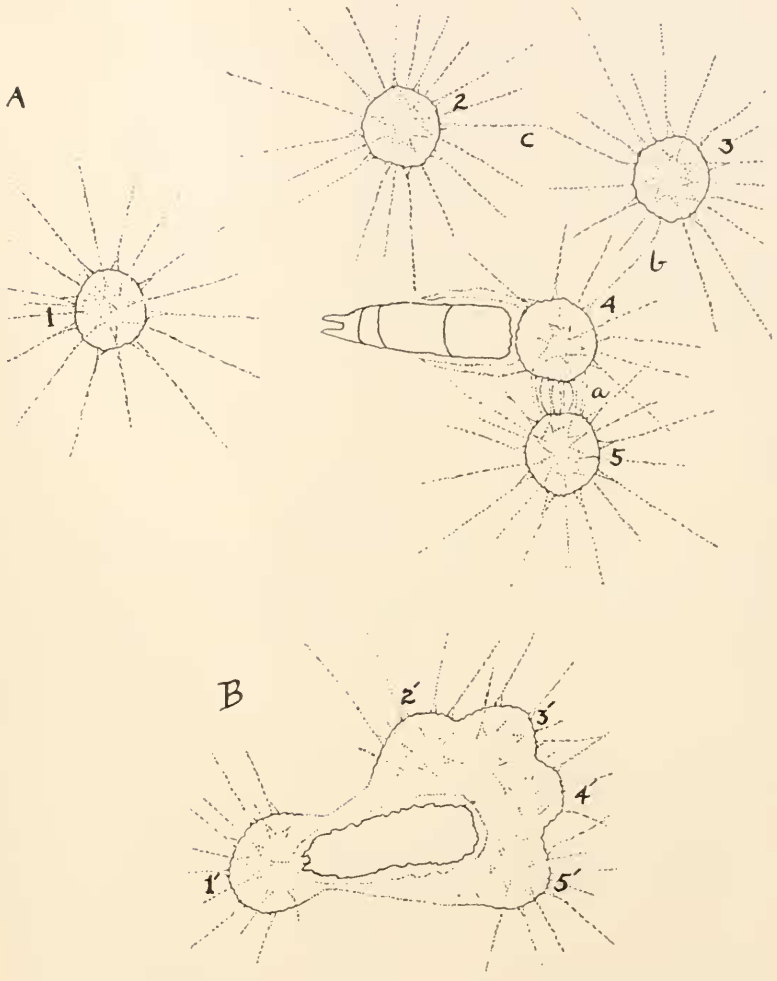

FIG. 3. *A*. Five *Actinophrys sols* later to be involved in the capture and ingestion of a rotifer. Specimens 4 and 5 already show advanced coöperation, *a*. Specimens 3 and 4 show the inception of coöperation through the fusion of two of their slender rays, *b*. Specimens 2 and 3 are also involved in the coöperation through the fusion of two rays at *c*. Specimen 1 is yet independent.

B. Same group showing progress made within two hours. Complete ingestion has been accomplished and some digestion has ensued. 1′, 2′, 3′, 4′, and 5′ shows the position taken by 1, 2, 3, 4, and 5, respectively, of Fig. 3 *A*. (× 260.)

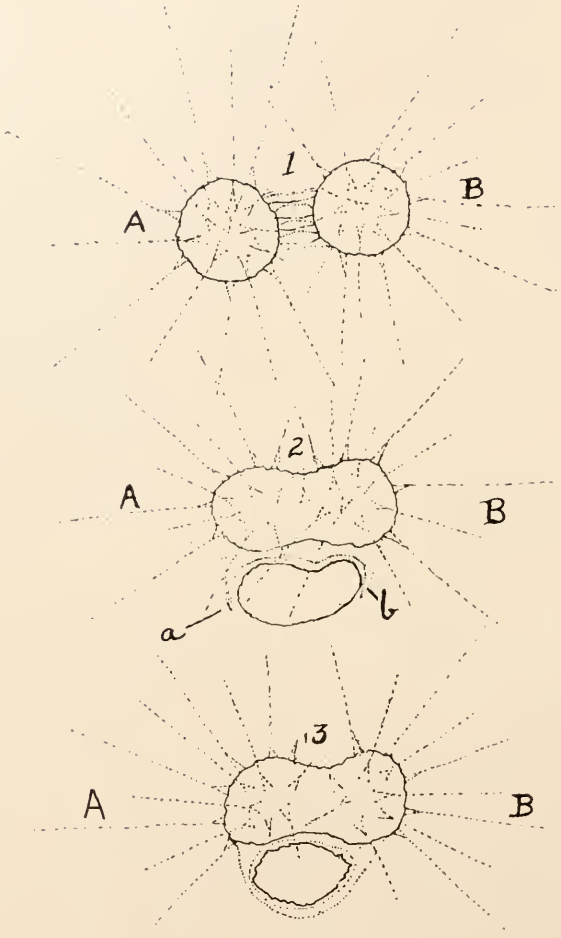

FIG. 4. Sketches illustrating the fusion, also, the coöperation of two *Actinophrys sols* in the capture and ingestion of a *Colpidium*.

1. Pseudopodial fusion in the union of the two individuals.

2. Coöperation of the same two individuals in ingestion of *Colpidium* by one individual, *A*, sending out *a* and individual *B* sending out *b* to form in common one large food-engulfing pseudopod.

3. Same specimens one and one half hour later. Prey engulfed by fusion of pseudopods *a* and *b* about it. ($\times$ 325.)

the various types of objects it may easily be seen that *Actinophrys sol* feeds chiefly on the smaller ciliates and on the flagellates. The experiments with moving animate objects show that certain of these objects are eaten more readily and in greater numbers than are others. Difference in the size and vigor of the different food objects, no doubt, plays an important rôle in causing this discrimination. However, it is not probable that difference in size and vigor alone is the only factor causing the discrimination noted. The experiments with moving animate objects also show that such objects are not embraced intimately by the food-getting pseudopods of *Actinophrys sol;* but on the contrary, the moving animate objects, as a rule, are loosely or subtly embraced by the food-getting pseudopods.

Summary.

1. *Actinophrys sol* is essentially omnivorous.

2. Certain inanimate objects are ingested more often than others. Some inanimate objects apparently are not ingested at all.

3. Motionless animate objects are accepted, but not in appreciably greater numbers than inanimate objects.

4. Many free-swimming ciliates and flagellates are readily eaten, but certain ciliates apparently are not taken.

5. A degree of selection or discrimination was exercised between different food objects in every class of materials used.

6. Large objects of prey may be taken in by temporary colonies of *Actinophrys sol* which are formed for the purpose of taking in such objects.

7. As a rule, motionless animate objects and inanimate objects are closely embraced by the ingesting pseudopod; objects which struggle while being ingested are subtly embraced.

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THE HONEY-GATHERING HABITS OF *POLISTES* WASPS.

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Wheeler, in his valuable paper on "Vestigial Instincts in Insects and Other Animals," * gives a résumé of what is known as the honey-gathering propensities of wasps, principally those of the tropics. He also records his own discovery on November 3 of a nest of *Polistes metrica* in New York City, hanging from the eaves of a boathouse. This nest of a few dozen cells had four females, inactive from the cold, clinging to the comb, and six cells contained half-grown, much contracted but still living pupæ. There were small drops of colorless liquid in many of the other cells, and one of them was half full of this substance, which tasted like and proved to be honey of an agreeable flavor. These drops, he tells us, hung suspended in the angles of the cells, but were without any definite arrangement, and varied much in size. "This honey must have been collected some weeks previously from the autumn flowers and stored, now that nectar and insect food were no longer to be had, for the purpose of bringing the few remaining larvæ to maturity. The belated brood undoubtedly accounted for the presence of the female insects at so late a date."

Wheeler assembles the details of the observations on honey-storing by tropical wasps made by Lepeletier, Rouget, Marchal and others, and then goes on to show that in the northern species this habit of honey-storing is a vestigial instinct, and is called into play only when the insect is facing unusual conditions. To use his exact words: "The great quantities of honey collected by the tropical wasps are, of course, stores of provisions for the winter, for, as the von Iherings have shown, many of the species, unlike the northern *Polistes*, do not abandon their nests on the approach of the unfavorable season and start new ones in the spring, but continue to add to their combs and keep on raising their brood

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throughout the year. The naturalists are unquestionably right in deriving the conditions seen in our northern *Polistes* from those of the tropical species. There can be no doubt that *Polistes* has extended its range into North America and Europe since the close of the glacial epoch. The storing of honey for the winter has been discontinued, and the life of the species has been saved by a new set of adaptations involving the abandonment of the nest, the temporary suspension of the breeding instincts, and the hibernation of a small number of fertilized females. The drops of honey occasionally stored in the nests are all that remains to point to a once very important means of tiding over the flowerless season and preserving the life of the individual colony. Rouget, Brongniart and myself have observed this vestigial instinct only in the autumn, and this would seem to be the most likely time for the feeble display of the old habit.

"Not only has the honey-storing instinct of our northern *Polistes* been reduced to a feeble and useless vestige by the adaptation of this insect to life in a temperate zone, but the nest-building instincts, when compared with those of the allied tropical wasps, show unmistakable signs of similar degeneration."

This interpretation was to me highly fascinating, for I too had occasionally seen similar drops of this clear, honey-like substance attached to the walls of the cells of *Polistes pallipes*. Other things crowded out further investigation of this point until early in 1920, when the above-mentioned paper fell into my hands, and I decided to look fully into the matter, with the following results.

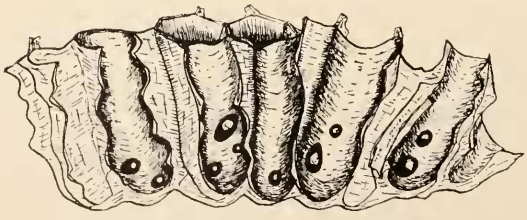

FIG. 1. A cross section of a nest of *Polistes pallipes*, showing globules of honey in the cells. (Exact size.)

Investigation No. 1.—On June 16, 1920, twelve nests of *Polistes pallipes* were found in the old buildings at Wickes, Mo., and one nest was far in among the branches of a closely foliated cedar tree. None of these cells had workers, but each nest was

presided over by the queen. A close examination was made of each nest, and the twelve in the buildings showed no evidence of having drops of this jelloid substance in their cells. The one nest out in the cedar tree, however, gave positive evidence. This was a nest of fourteen cells, with the four central ones filled with half-grown larvæ; these cells contained none of the jelloid material, but the ten surrounding cells each contained an egg and from one to three drops of this material glued to the angle of the cell. Some of the globules were quite large. An idea of the relative size when the terms large and small are used may be gained from Fig. 1, which illustrates the globules, exact size. The substance had a decidedly sweetish taste. This was an absolutely newly made nest; there was consequently no possibility that this material could have been left over from the preceding year in an old nest. The twelve nests which gave negative results also contained eggs, so of the thirteen mothers which worked in this neighborhood, only one provided this material for her young.

Investigation No. 2.—The one nest containing the globules in the previous study was taken; the other twelve were undisturbed, with the idea of studying them later. By mid-summer (July 21), the workers had matured and covered the nests, so that observations from day to day were impossible on account of their inaccessibility, the ferocity of the occupants and the poor light. With so important a problem, I could do nothing but knock the nests down with a stick, and after the infuriated wasps had flown back to the place where the nest had been, harvest my booty.

In this study, thirteen nests were taken and carefully examined; ten of these were nests of *Polistes pallipes*, one was *P. variatus* and two were *P. annularis*. Every nest except one of this lot gave evidence more or less abundant to show that all of these three species store drops of this "honey" in their cells containing eggs or very tiny larvæ. Throughout this study I have never found these drops in cells containing larvæ more than a few days old. The data herewith give the details for the thirteen nests, all taken on July 21, 1920.

In the foregoing table, the first ten nests were *P. pallipes*, "k" was *P. variatus*, and "l" and "m" were *P. annularis*. These few nests show that all three species follow this habit of supplying

Nest.	Cells Only Begun.	Cells Cont. Eggs.*	Cells Cont. Honey.	Cells Cont. Larvæ.	Cells Cont. Pupæ.	Total Cells in Nest.	Remarks.
a.....	—	12	1	—	—	12	
b.....	—	30	0	—	7	37	
c.....	—	15	14	6	10	31	From 1 to 3 beads of various size. Note: much honey but no larvæ.
d.....	6	30	25	—	—	36	
e.....	—	12	3	5	5	22	
f.....	—	24	24	3	9	36	One to 3 drops of various size.
g.....	—	26	14	5	7	38	
h.....	—	75	49	13	13	101	Drops varied in size, number and consistency.
i.....	—	36	32	6	18	60	
j.....	—	61	49	7	10	78	From 1 to 4 beads, many of them very large.
k.....	—	20	20	10	14	44	From 1 to 3 globules of various size.
l.....	—	22	2	13	4	39	
m.....	—	38	1	23	41	102	
13	6	401	234	91	138	636	

* This signifies eggs or newly hatched larvæ less than a day old.

their young with this honey, but of course from such limited data we can have no idea whether any one species is more constant in this habit or furnishes more profuse supplies. The totals show at a glance how common is the occurrence of this food in the egg-cells of wasps even at the middle of the summer season. Out of the 407 cells containing, or about to contain an egg, 234 of them or almost 58 per cent had globules of this honey-like material. The actual occurrence of the honey was probably even more frequent than the numbers indicate, for the reason that the first-day larvæ were included with the eggs, and in some cases they had doubtless already devoured their rations before I counted them.

Investigation No. 3.—In the foregoing experiment, I removed and carried home the thirteen nests of *Polistes,* leaving the adults behind. Three days later, on July 24, I returned to the scene to see if they had rebuilt the nest, and especially to find if, without the stimulus or the aid of the larvae present, they would supply these drops of sweet food in the new cells. The details of their behavior in rebuilding are given elsewhere, but here I record that of the nine colonies which did rebuild (eight of *P. pallipes* and one of *P. variatus*) each nest had from fourteen to twenty-two cells, all very shallow at this stage, but each and every cell contained its globule of jelly along with its egg.

Investigation No. 4.—Records of two nests taken at Wesco, Mo., on August 3, gave the following data. The first was a nest of *P. variatus*, which contained thirty-five cells with eggs, sixteen cells with larvæ and forty-four with pupæ, and had about a dozen adults on the nest. This large nest gave absolutely no evidence of the presence of the honey. The other was a nest of *P. pallipes*, which was in all probability the second one made by this colony, since at this late date it had only small, newly-made cells which would hardly hold a half-grown larva, and there were no cells large enough to have harbored the five adults which were then on the nest. These shallow cells, fifty-two in number, gave no evidence of having a supply of honey, although about one half of them contained eggs.

Investigation No. 5.—Since I had removed all of the accessible nests at Wickes, the next scene of operations was at Meramec Highlands, west of St. Louis and about thirty miles distant from Wickes. These nests were gathered about two weeks later, on August 6, 1920. In this collection, fourteen nests (all *P. pallipes*) were knocked from the walls and eaves with long poles and carried

Nest.	Cells Just Begun.	Cells Cont. Eggs.	Cells Cont. Honey.	Cells Cont. Larvæ.	Cells Cont. Pupæ.	Total Cells.	Remarks.
aa....	10	35	35	—	3	48	All ten new (eggless) cells, and 25 of the 35 cells with eggs contained honey.
bb....	—	23	8	32	14	69	
cc....	—	10	6	25	28	63	
dd....	—	11	6	21	22	54	
ee....	10	20	25	32	45	107	All 10 new (eggless) cells, and 15 of the 20 cells with eggs contained globules.
ff....	6	23	25	18	7	54	Again, all 6 empty cells contained drops of honey.
gg....	—	14	10	38	20	72	
hh....	—	54	35	27	20	101	Some of the globules immensely large.
ii....	—	12	10	14	3	29	
jj....	—	10	10	4	3	17	
kk....	28	11	39	8	21	68	All new empty cells and all cells with eggs supplied. 1 to 4 drops per cell, some very large.
ll....	—	57	56	16	25	98	
mm...	—	36	15	36	65	137	
nn...	3	4	3	—	—	7	New nest with no workers.
14....	57	320	283	271	276	924	

home for examination. The data from these will also be more convenient if presented in tabular form.

In this table we find that out of 377 cells containing or ready for an egg, 283, or about 75 per cent. of them, contained drops of this honey-like substance.

The next spring, the following additional experiments were made.

Investigation No. 6.—On this date, April 28, 1921, I found forty-two small nests of *P. pallipes* which had only recently been begun by their queens. In only one cell of all these nests did I find one drop of this honey suspended in the angle of the cell. This one drop is sufficient to show that very early in the nesting season the queens sometimes store this substance. At this season the queens were not so bellicose, so instead of knocking down the nests, at intervals for examination I climbed up to the nests and, with the aid of a flash-light, examined the nests and contents *in situ.*

Investigation No.7. On May 13, twenty-nine nests, with the number of cells varying from ten to eighteen, were minutely examined, and fourteen were found to contain drops of honey. Nest No. 1, with thirteen cells each containing an egg, had a drop in each of two cells. One cannot say that this had been placed there for the young about to hatch, for these two cells were adjacent and were at the margin of the nest, whereas the first eggs to hatch are as a rule in the center of the comb; in this case the eggs in the two center cells were even then much inflated and ready to give forth their life. Nest No. 56 had ten cells all containing eggs, the two central ones large and ready to become larvæ. It was in precisely these cells, that the drops of honey were found. Nest No. 15 comprised fourteen cells having eleven eggs and three larvæ in the center cells. Two other cells adjacent to these had each a drop of honey. The larvæ were several days old, and no evidence remained in their cells to tell whether or not they had had this food in their infancy. The eggs provided with honey were inflated and ready to hatch next. Nest No. 22 had ten cells each containing an egg; the largest of these, already swollen with its embryo, was provided with a drop of honey in its cell.

Thus we see four nests out of twenty-nine at this early date containing honey-drops; I suspect that the larva, upon emerging, gets

its first meal from this food, and in three of four of the cases observed on this date this material had been placed in the cells containing first-laid eggs, the larvæ from which would be first in need of food. However, since only about one-eighth of the nests examined contained honey in even a part of their cells, it seems very likely that some of the young lack the advantage of this food, or at least do not have it waiting for them before their birth. The most interesting bit of evidence that this day gave forth, however, was the one cell, which on April 28 had contained a drop of honey, on this date, May 13, contained a small larva but no honey! The period of incubation in this species has elsewhere been found to be a little more than two weeks, so it is not surprising if the mother cannot estimate exactly the date of the need of food for each of her young.

Investigation No. 8.—The accompanying table summarizes the details of the observations on twenty-seven nests on May 27 and 28, 1921. At this time, when the nests were larger and contained more life, I found less of the honey; it occurred in only three of the twenty-seven nests, and as usual only in the cells containing eggs; out of 142 cells only 13 had drops of honey. It seems at first puzzling that a mother would supply this extra provision to some cells containing eggs, and not supply it to all, and that if one mother is so provident, not all are likewise. All were *pallipes*.

I previously formed the hypothesis that all young, when they first hatch from the egg, must be fed this material probably predigested by the mother; that frequently she is on hand at the time and administers the dose personally, and that sometimes she gets an over supply or gathers it before the young are hatched, in which event she relieves herself of it by placing it in the angles of the cells containing the eggs which will soon hatch. The following notes will to a degree bear out the first part of this hypothesis.

While restlessly waiting for a belated train at a rural flag-station at 10 A.M., I fell to watching a queen which had charge of a nest built on the wall of the station shelter. I noted that repeatedly she inserted her head deep into two cells and kept it there far out of sight for periods of three to seven minutes. I knew that trophallaxis occurs between the larvæ and the workers, but this queen had crowded her body so deep into the cells as to completely

Nest.	Cells Just Begun.	Cells Cont. Eggs.	Cells Cont. Honey.	Cells Cont. Larvæ.	Cells Cont. Pupæ.	Total Cells.	Remarks.
51....	—	3	—	13	—	16	
69....	—	11	—	5	—	16	
9A..	—	5	—	6	—	11	
11....	—	2	—	11	—	13	
12....	—	7	7	7	—	14	Honey with all the eggs; none with the larvæ.
1....	—	4	—	10	—	14	
2....	—	10	—	2	—	12	
3....	—	—	—	16	2	18	
13....	—	8	—	10	—	18	
8....	—	2	—	7	—	9	
35....	—	7	—	8	—	15	
34....	—	5	—	10	—	15	
31....	—	6	—	12	—	18	
54....	—	18	6	14	—	32	Honey all in egg cells; this nest had 3 queens.
14....	—	2	—	10	—	12	
15....	—	—	—	10	2	12	
21....	—	4	—	4	—	8	
17...	—	6	—	6	2	14	
58....	—	1	—	10	4	15	
59....	—	2	—	10	1	13	
20....	—	2	—	10	—	12	
23....	—	3	—	11	—	14	
21....	—	—	—	10	—	10	
72...	—	4	3	—	—	4	
73....	—	12	—	—	—	12	
74....	—	12	—	—	—	12	
22....	—	6	—	8	—	14	
27....	—	142	13	220	11	373	

cover the thorax; this indicated at once that the cell must contain only a very small organism, and thus the present process could not be trophallaxis. Presently she removed her head from the cell from time to time and went about examining three other cells which contained eggs; six other cells nearby contained good-sized larvae, but these she ignored. Her attention was always centered about the two cells whose contents I could not see; whenever she left one or the other of these two cells, she would poke her head momentarily into each of the three egg-cells and then go back to the others. When visiting the egg-cells, she would always vibrate the antennæ, bring them close together and point them in front of her head as she entered, insert the head for an instant and withdraw, repeat the process exactly for the other two, and then return and creep deep into one of the mysterious cells for several minutes. When she withdrew from either of these, she moved her mandibles

in a significant manner, but very different from the "licking-her-chops" manner of the workers after they take the liquid from the larvæ, for in the latter case not only were the mandibles in motion, but also the labium, palpi, etc., were actively registering satisfaction or function.

The approaching train brought the observations to a sudden close. Hastily I pulled out the mother wasp and threw the gleam of the flashlight deep into the cells, and lo! in the far corner of each lay a tiny larva, hardly larger than an egg! I could solve the mystery of her long stops in these two cells only by concluding that she was feeding them with the material from her own mouth, since I was sure she brought nothing to the cells in her jaws. I pondered on the number of times she had poked her head inquiringly into the three cells containing eggs; then I realized that her frequent visits here were probably for the purpose of ascertaining if they had yet hatched or were ready for her attention. The larvæ she did not need to mind just now, but the new-born twins and the eggs had all her attention.

My most recent observations have led me to believe that the queen does not deposit this honey with the intention of making a self-feeder for the larva, so that when the egg hatches the larva has only to thrust its head into it and imbibe, but rather that she stores it here temporarily where it will soon be needed and where she can easily administer it to the waspling as soon as it is ready. The ground for this conclusion is the fact that these drops are almost always placed far below the egg in the cell, or on the opposite wall, so that it is quite impossible for the new-born larvæ to reach them. I have seen dozens of young larvæ in early spring nests which had been brought into the laboratory, die with large drops of honey before their very eyes but out of their reach; since the tail of the larva is glued to the wall, its progression to the drop is impossible. In contrast to this, I have seen a few larvæ survive on this substance when it was artificially placed near their mouths. I have also fed them on that taken from other cells; they ate it readily when served to them on a pin-head. I have also assisted larvæ to get this food by pressing the opposite wall of the cell toward them, bringing the drops nearer to their mouths; and in the past two years I have actually seen two cells in which the drops of

honey were placed very near to the larvæ, and they were stretching down and actually imbibing the substance.

The above instances indicate that the newly-hatched larva gets its first meal from the mouth of its mother. This of course can be independent of the storing of the honey as we find it in some cells, but it might point very definitely to the origin of this habit. How logical it seems for all the mothers to feed their new-born young upon predigested food from their own crops, and how possible it could be for the crops to be filled too full or filled too soon. In that event the next logical step would be for the mother to dispose of this surplus by placing it in the cells where she would normally get rid of it at an early date. While one does not wish to speculate too far, one can also see the possibility of another pretty adaptation, in that mothers with only a few offspring at that stage can feed the delicate food to them personally, but when the young are too numerous and the mother's attention must be divided, food may be supplied to them in advance—a habit well known among the solitary wasps. To go further, how possible it is for a social species to show vestiges of a habit of the solitary species,[1] *i.e.*, to supply the egg with food in sufficient amount to carry it to maturity.

Investigation No. 9.—On June 21, 1921, I examined the contents of 20 nests of *P. pallipes*. The egg-laying was over for the time being. It seems that the queen of this species spends her time at this season in caring for the young, and not in adding new cells and depositing more eggs. It was then thought that perhaps the building activities would be resumed after the workers emerged. Only six of the twenty nests contained a very few eggs; hence one would expect proportionately fewer drops of honey in the cells. That is precisely the condition that I did find; in all of the twenty nests examined, only one was found containing one drop of this material; this was in an empty cell of a nest which contained five cells with larvæ, one with an egg, and five empty cells.

Investigation No. 10.—This paragraph relates merely the accidental discovery of honey in a nest of *P. variatus*. This nest was in an inverted, rusty teakettle in a city dump-lot. My attention was attracted to it by seeing the mother enter through a crack near

[1] Bouvier ("Psychic Life of Insects," p. 335) says that the social insects have developed from solitary ancestors.

the bottom, where she was obliged to alight and creep under the vessel. By carefully lifting the whole outfit I could observe the contents. The nest was a strong one of about twenty cells with eggs and larvæ. Here *P. variatus* had again demonstrated her fondness for low situations by building her nest in this unusual site near the earth.

On the morning of May 17, I surprised the mother by lifting up the old teakettle to the level of my eye for examination. She was for a time too much bewildered to move—and the sight that met my eyes affected me likewise, so it was some time before I recovered myself to set it down again. Each one of the eight cells containing eggs had also a large globule of this shining, transparent liquid, so large that it half filled the cell. The next morning, upon similar examination, I found the globules much reduced in size and more viscous in nature. The mother was then on the nest, where I had evidently caught her in the act of bringing in more of it, for in her jaws was a large, shining globule, surprisingly clear at this stage. Three days later these large, watery globules were still present. The mother was again on the nest, but this time she was chewing a yellow ball of caterpillar meat. This was undoubtedly her own food, since I could see no larvæ in the cells to which she could feed it. Thus the evidence indicates that from the very first, *P. variatus* stores a sugary liquid, which with evaporation condenses into a jelly-like substance; that she adds to the store from time to time, and eventually the residue is in the form of sticky balls or drops. It is interesting also that for her own food the queen sometimes catches caterpillars and brings them home to her nest to devour them, as a variation from her nectar diet.

Two weeks later these cells were again seen to contain large globules of liquid, like huge, yellow dew-drops. The eggs had all hatched; the larvæ were still tiny. This set me to wondering whether, in a large, dry field where there was no possibility of getting water, the mother did not gather drops of dew each morning and store her supply of drink for the day. The next day I was greatly disturbed to find that the ubiquitous small boys, who had been spying on my strange behavior in the dump-lot, had discovered the quaint nest and destroyed it.

Investigation No. 11.—At the end of May, 1921, only a few small drops of honey were found in a large number of nests, to be exact, only three in twenty-seven nests. In May, 1922, extensive examinations showed that this year the honey content was so abundant as to be very conspicuous in a large proportion of the nests; twenty-five of the thirty nests each contained the drops in many cells, and larger than usual in size. These were only in the egg cells and the new cells; none were in the cells with larvæ. Just in what way this year afforded conditions which produced this excess, I can only surmise. It does seem probable that the abundance of flowers or the climatic conditions would be factors influencing the supply. On cold, rainy days the queen seldom leaves the nest.

Glancing at the data as a whole one sees at once that this honey-storing habit is much more common in these three species of *Polistes* than was generally thought to be the case. Furthermore, it is probably not a local condition, since the collections from two places thirty miles apart showed this characteristic about equally well established.

Early in the course of this investigation I thought that this substance was the product of the large larvæ. I had observed the workers gathering the saliva-like secretion in large amounts from the mouths of the larvæ, and knew that the adult wasps seemed to regard this as a delicacy and enjoyed it as food. So, since the color was similar, I suspected that the worker, after the material had perhaps undergone some chemical change in their gullets, would place it in the cells, so that the new-born waspling would find a supply of the most delicate food for its first meal.

Soon the evidence from the observations began to militate against this theory. The results of the third group of observations left my belief well-nigh untenable, since the groups of adults whose nests were taken away built clusters of new cells at once, and within three days had the majority of these tiny cells well supplied with this substance. This was done after all their own large larvæ had been carried away. Of course the possibility remains that the adult workers, of which there were plenty at that time, might already have had their crops or gullets full of this, gathered from the nestlings when the catastrophe came, and

that they retained this until they could deposit it in the new cells, but this supposition seems far-fetched. Also in Investigation No. 2, nest "*d*," we find a nest entirely without larvæ, yet twenty-five of the thirty cells of the egg stage contained honey. Surely the workers would not venture to go to other nests and rob the larvæ of their salivary secretion; moreover the queens had been storing it at a time when there were no larvæ from which they could gather it. In consideration of these points I felt compelled to doubt or even abandon the idea, and turn to the hypothesis that these drops are vegetable matter, gathered from the flowers and fed to the tiny larva or stored in the cells awaiting its use, like the honey stores of other Hymenoptera. It is unfortunate that we must thus abstractly speculate—that no one has yet actually observed the wasps collecting, making or storing this material, so that we may know with certainty just how it is done and may figure out the relation of the habit to the life or even to the history of the insect. Here, however, I can only continue to give the evidence derived from the foregoing data, in the hope that it may pave the way to conclusive observations in the near future.

In several of the nests observed, especially the new nests described in the third lot, we have in all probability a very definite clue to the nature of the material in these drops. In all nine nests in the third lot, which we knew to be very new, and in other nests where there was evidence that the cells had been made and stored very recently, the globules were exceptionally large and of very watery consisteney. This gave rise to the idea that the material is probably similar to that gathered by bees and that it must likewise be subjected to evaporation, as is the nectar in the bee-hive, after which it retains the form of little, firm beads. In the large nests the comparative age or the chronological order of the cells could not be definitely determined, but one small, new nest lent its evidence to this theory. The three central cells each contained a drop, one of them condensed and bead-like, the two others in an intermediate condition. The last cell, a new and incomplete one, contained a huge drop of very watery substance. I placed the entire nest in a tiny tin box to take it home for study, but the large drop was scattered or absorbed by the paper walls en route. This little nest seemed thus to show the natural evolution of a

drop of this gelatinous material, from big, watery sweetish drops in the newest cell to smaller, waxy beads in the older cells.

Thus the material from distinct localities shows that this honey is used much more generally than investigators have heretofore suspected. Furthermore, it is present not only at the end of the season, provided to tide over some tardy larvæ, but at all times throughout the season, less frequently during the spring when the queen alone tends the nest, and most frequently and in greater abundance toward the end of the summer. Whether this greater abundance in August is due to a larger supply of flowering plants, or possibly to the greater leisure and number of the workers, or whether they actually gather this material to feed certain larvæ in order to develop queens, remains for further investigation to determine. The frequent lack of this food in the earliest cells of the season might point toward this latter suggestion.

In concluding one would say of Wheeler's theory, that the honey-gathering habit in *Polistes* is a vestigial instinct which is called into play to carry over tardy larvæ, can hardly apply to *P. pallipes*, *P. annularis* and *P. variatus*, since in these species the drops of honey are to be found at times throughout the season. The evidence seems to show that this instinct is not vestigial but functional, and is probably necessary for the survival of the young.

The watery consistency of the drops might give a clue to a solution of the evolution of the habit. We know *Polistes* are heavy drinkers, and probably they give water to the young, as do honey-bees. It might happen that for convenience this water was disgorged on the walls of the cells; by and by a portion of nectar from their throats was accidentally added, and then with evaporation there remained but a small, transparent globule.

Sharp says,[1] in speaking of the habits of social wasps; "... the eggs soon hatch and produce larvæ that grow rapidly; the labors of the queen wasp are chiefly directed to feeding the young. *At first she supplies them with saccharine matter,* which she procures from flowers or fruits, but *soon gives them a stronger diet of insect meat.* ... The hornet is particularly fond of the honey-bee." Unfortunately Sharp does not give us the specific name of the wasp upon which he bases this statement, but that this is based

[1] "Insects," Pt. II., p. 84. [Italics are mine.]

careful observations on some species and not upon mere theory is corroborated by my notes on *Polistes*.

To theorize upon the phylogenetic position of this habit is a small task, and evidently can be substantiated by direct observation. It seems probable that the flower-loving insects evolved from non-flower-frequenting species about the time of the appearance of flower-bearing vegetation. The flower-visiting Aculeates, the bees, were pollen- and nectar-gatherers. If we assume that the bees and wasps had, 'way back in prehistoric time, the same ancestry, we can gather that, phylogenetically speaking, honey-gathering is an older trait in Aculeates than insect-gathering.

Here we have wasps that gather nectar for their own food and honey for their young, and super-imposed upon these traits we have the newer habit of insect gathering for the young. This honey-gathering is not a recent addition to their feeding instincts, but a vestige which proves their ancestry from the bees.[1]

A step further was made when probably certain bee-like, honey-feeding Aculeates back in geologic time, from scarcity of flower food or drought, or through pure "change of mind" or by accident, began to prey upon other insects that sought the same flowers, for their own food or for food for the young in their nests. Sharp tells us that the hornet is particularly fond of honey-bees, which suggests the thought to my mind that the change in habit from the use of flower products to meat may not have been so wide and abrupt as the lines would indicate. It seems so easily possible for certain ones to get a little additional food by licking one another's faces and mouths, just as we see *Polistes rubiginosus* doing today;[2] in time of scarcity of food this habit might easily be exaggerated even to the point of robbery or squeezing out the sweet liquid from the honey-crops, as Fabre describes in the modern behavior of *Philanthus*. From this stage it would indeed be a poor Aculeate that would not eventually learn to abbreviate

[1] I am aware that the opposite view is generally held, for Wheeler (*Scientific Monthly*, 15: 68–69, 1922) says: "The structure and behavior of the Sphecoids and Vespoids show that they must have arisen from what have been called Parasitic Hymenoptera, and the structure of ants and bees shows that they in turn must have arisen from the primitive Sphecoids or Vespoids."

[2] Paper soon to be published.

the work by making a perforation in the body wall to reach the nectar crop directly (just as Robertson describes for *Odynerus foraminatus* that bites an opening into the base of the flowers of *Pentstemon lævigatus*)[2] for the same purpose, or to carry home an entire insect, making it serve as a vessel to hold the honey, without any further trouble. This puncturing of the living honey-pot would lead to a taste for animal juices which we know now exists in certain Pompilids. Even if the habit of carrying this prey home had not by this time been acquired, it would be but a step to learn to carry it to their young after they had learned to enjoy it themselves.

An additional bit of evidence to indicate that the nectivorous habit probably antedates the carnivorous habit in these insects is that, as Sharp tells us and as we have seen in the observations just recorded, the mother feeds to the new-born young first a nectar compound and later, animal food. If we accept the principle that ontogeny recapitulates phylogeny, it is easy to find significance in the fact that the immature organism passes through the nectar-feeding stage before it arrives at the carnivorous stage. Among the surviving descendants of the primitive flower-loving Aculeates, some have digressed to the extent of finally evolving into carnivorous creatures with certain feeding traits that are vegetative.

In my opinion, the honey as I found it was merely stored honey that had been carried in the crop of the queen or the worker for the purpose of direct feeding into the mouths of the young, and this over supply was deposited for convenience on the cell wall. Since these drops are not present at all times in all cells with eggs, in the nests of the three species of *Polistes*, the evidence lends some merit to the theory that these drops are stored by law of supply and demand: a queen having many young larvæ to care for must needs fill the crops of the young directly from her own gullet and has no surplus to store in drops; on the other hand, when the eggs hatch at intervals, the mother has sufficient time during the period of watchful anticipation to store up a surplus. Another factor in regulating the number and size of the drops is the abundance of

[2] In order to get the nectar which she could not ordinarily reach, this wasp cut a hole in one side of the base of the tube with her sharp jaws and inserted her tongue; then she cut a hole in the other side and again inserted her tongue. (*Trans. Acad. Sci., St. Louis,* **5**: 569, 1891.)

flowers, and still another reason for the presence of so much in the latter part of the season may be that then the workers are also on hand to help bring it in.

I often wonder if the honey-storing habit in the honey-bee was not evolved in the same fashion. Is it not possible that originally nectar was gathered and the regurgitated material fed direct to the larvæ; that finally, when workers became abundant in the colony and more was brought in than could be consumed, they resorted to dumping it into cells without larvæ, and that this may have been the forerunner of the honey-storing habit? A contrasting trend of development may be found in the honey-gathering ants, *Myrmecocystus horti-deorum,* which do not eject this substance, and their distended abdomens as they hang to the roof of the honey-chamber are the actual store-houses of the honey.

The reader must bear in mind that the foregoing explanations of this phenomenon are only speculative, but I have tried to use as much logic as I could instil into the ideas. In this I take refuge under the justification of C. H. Eigenmann, when he says: "The imagination is in Biology as elsewhere the guiding spirit. The trouble is our imaginations are sometimes too heavily loaded with statistics, and at other times they fly without the balancing kite's tail of facts. The Palæontologists have contributed to speculative Zoölogy because their imaginations have been kept alive by bridging their numerous gaps, and because they have not been hampered by too great a wealth of material."

ENDODERMAL FLAGELLA OF *HYDRA OLIGACTIS* PALLAS.

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The subject of flagella upon the endodermal cells of hydra was first opened prior to 1871 by Hatchek, who figured a single tapering flagellum upon each cell, all of which he considered similar. F. E. Schulze ('71), figured a single flagellum to each cell of an optical section of a tentacle. Kleinenberg ('72) in transverse sections of the living animal observed one or more flagella in connection with several cells and claimed that the flagella were not fixed structures but that they could be protruded and retracted again, while at the same time the cells sent out pseudopodial processes. T. J. Parker ('80) saw one, two, or three cilia on cell after cell. Because of this observation he drew the inference that the endoderm was ciliated throughout. K. C. Schneider ('90) distinguished at least six types of endodermal cells; but he stated that only three of these types bore flagella. The digestive or epithelio-muscular cells usually bear two flagella, which project into the enteron. The glandular cells bear two or three flagella. The sensory cells bear one, two or no flagella. Schneider gave us, therefore, the first proper description of the flagellated condition of hydra's endoderm. Hadzi ('09) did not carry the knowledge of the flagellated condition of the endoderm beyond Schneider. I have been able to corroborate the work of Schneider and to carry his observations a step further by the use of the following method.

Hydra oligactis was macerated by Mundie's ('26) method. In this case a hydra was placed upon a slide and the water drawn off until but a film remained covering the polyp. The slide was then placed over the mouth of a bottle containing Looper's fixing fluid (made up of equal parts of 95 per cent. alcohol, glacial acetic acid and 40 per cent. formalin [1]). At the end of eight or ten minutes

[1] This method was developed in this laboratory by Dr. J. B. Looper.

the polyp was rinsed in one or two drops of water, as much of this water as possible drawn off and a drop of Gramm's iodine solution added. The polyp was teased into fragments with needles, a drop of 40 per cent. glycerine added and a coverglass applied. The fragments were then examined under the oil immerson objective. Being in glycerine, the cells could be preserved for a relatively long time, a month or more. During this time the flagella persisted and the cells did not deteriorate. The tissues may even be further treated. For example, if the iodine fades, more iodine may be drawn beneath the coverglass. I have also carried one per cent eosin-licht gruen (95 per cent. alcoholic) solution beneath the coverglass and have, in this manner, stained nuclei, food vacuoles, flagella and other details well. In passing it may be stated that the use of the licht-gruen solution brings out conspicuously the pseudopodia of the epithelio-muscular cells of the endoderm.

The epithelio-muscular cells are columnar. A myoneme runs at right angles to the polyp's axis through their broad bases. The distal end bears one or more flagella. There are many food vacuoles in epithelio-muscular cells from well-fed specimens. The secreting cells of the general endoderm are club shaped with the smaller end directed toward the mesoglea. The distal end bears one or two flagella. The distal half of the cell is much vacuolated and in well-fed specimens these vacuoles contain darkly staining material called by Schneider, "Sekretballen." The basal end is darkly granular and bears no myoneme. The sensory cells are of the tall columnar type—almost filamentous. Each contains an ellipsoidal nucleus. These cells bear at their bases slender nodulated processes similar to those figured by Hadzi, in Table II., Fig. 7 and 8. In this type of cell we encountered a flagellum as did Schneider (Fig. 3).

I now come to the point at which my work goes beyond the work of Schneider upon the flagella of hydra. Some of the investigators describe the flagella as being tapering protoplasmic processes. Schneider shows them to be slender and of uniform caliber from base to tip. He does not, however, show a structure that is typically found associated with a flagellum, vis., a blepharoplast. All of my preparations show the flagella of epithelio-mus-

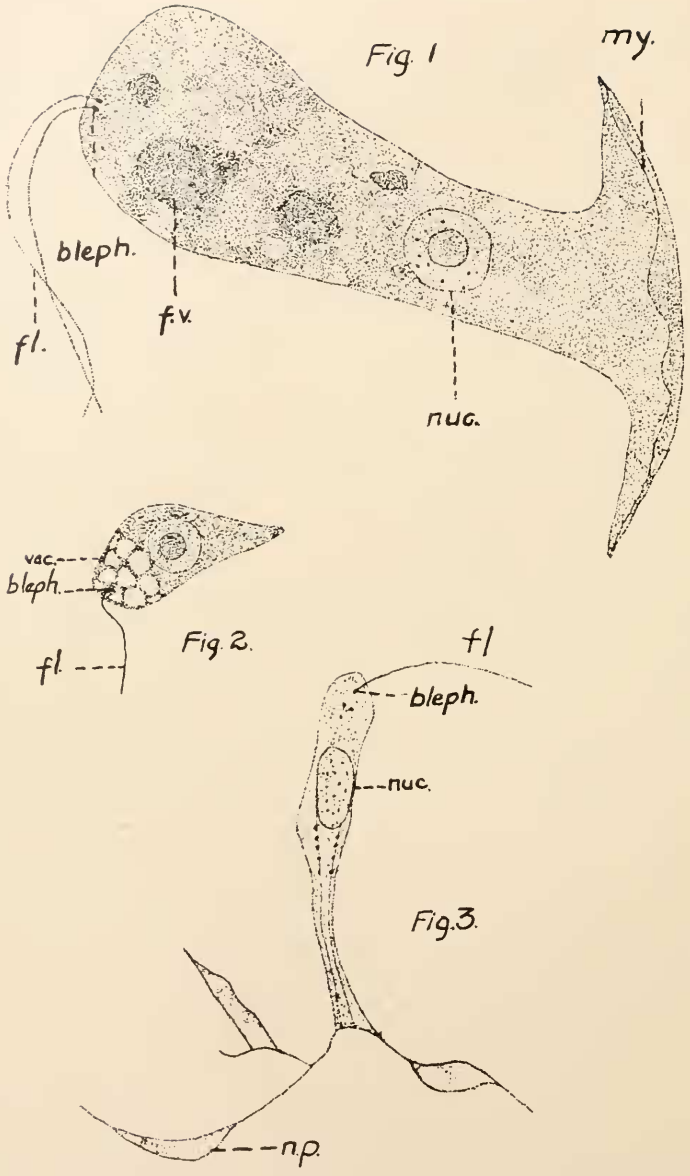

FIG. 1. Epithelio-muscular cell; *bleph.*, blepharoplast; *fl.*, flagellum; *fv.*, food-vacuole; *my.*, myoneme; *nuc.*, nucleus. × 1000.

FIG. 2. Secreting cell.

FIG. 3. Sensory cell; *np.*, nodulated process.

cular cells, glandular cells, and sensory cells to be associated with a blepharoplast. My Fig. 1 is of an epithelio-muscular cell that shows two flagella each extending into the cell's cytoplasm and ending upon a blepharoplast (Fig. 1, *bleph*). Fig. 2 shows, likewise, that in a secreting cell the flagellum enters the cytoplasm and ends upon a blepharoplast. Finally Fig. 3 indicates that the flagellum of a sensory cell enters the cytoplasm and terminates in a blepharoplast.

Summary.

The cells of hydra's endoderm—epithelio-muscular, secreting, and sensory—are flagellated. The flagella are typical in that they are non-tapering lash-like processes which terminate in blepharoplasts.

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A NEW HISTOLOGICAL REGION IN *HYDRA OLIGACTIS* PALLAS.

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Three anatomical regions are to be recognized in *Hydra:* (1) the oral end bearing tentacles, peristome and mouth; (2) the middle region; and (3) the basal region. In this oral region Kepner and Hopkins ('24) described, in detail, the peristomal endodermal glands that lie about the mouth. They also demonstrated the presence of sphincters at the bases of the tentacles. These sphincters operate against pressure from the enteron towards the tentacles but not in the reverse direction. The histology of the middle region has recently been intensively studied by Hadzi ('09). In this region he finds three types of cells presented by the endoderm: (1) epitheliomuscular cells, that are capable of ingesting small food-particles; (2) gland-cells, which lay down enzymes upon larger masses of food within the enteron; (3) (3) sensory cells. Hadzi, in this respect followed closely Schneider ('90), ('02) who had seen that each of these types of cells bore one or two flagella. Burch ('28) has carried this one step further in demonstrating that the flagella of the three types of endodermal cells arise from blepharoplasts. Not sufficient attention has been given, as yet, to the peculiar cells that lie laterally disposed in the basal region of *Hydra.* These cells are highly vacuolated and larger in calibre than are the endodermal cells of the middle region. In addition to these features, the endoderm of this region is peculiar because of the absence of gland cells. It is not yet clear to us that these cells bear flagella.

The lateral ectoderm of the basal region of hydra does not differ from that of the middle region. The ectoderm of the basal disc, however, has long been known to be peculiar. Korotneff ('80) wrote "Die Epithel-Muskelzellen des Fusses unterschieden sich von den ubrigen Ectodermzellen. Sie besitzen eine cylindrische Form, enthalten eine stark lichtbrechende Fibrille und zeigen in ihrem oberen Drittel eine gleichfalls stark lichtbrechende mucose Ausscheidung, durch welche die Anheftung des Thieres an fremde

Korper bedingt wird" (s. 165). We have not gotten beyond this early interpretation of the histology of the ectoderm of the basal disc.

With reference to the endoderm of this basal disc, however, we have something to add.

It had been noticed in this laboratory for sometime that hydras are sometimes found at low levels in aquaria with small gas-bubbles attached to the basal discs. Mr. George Dare made a careful series of observations upon isolated specimens and found that this gas was developed at the basal disc of hydra independent of the presence of bacteria or other organisms and of temperature changes. We then undertook the histological study of gas-elaborating specimens. We were able to make longitudinal sections that involved the complete or unbroken wall of the gas-bubble. This was done by gently transferring a hydra, that had a small bubble at its end in a drop of water to a slide. The slide was then placed upon a pinch of salt, that lay upon a block of ice, in such manner that the drop containing the hydra lay immediately over the salt. This resulted in rapid lowering of the temperature of the hydra. When ice crystals began to form about the margin of the water in which the hydra lay, it was flooded with Bouin's fluid. Thus the specimen was fixed and sectioned. The sections show the wall of the gas-bubble to be fixed to the marginal cells of the basal disc (Fig. 1, *g*), and the general or central cells to be in contact with the lumen of the gas-bubble, there being neither secretion material, bacteria, nor other substance lying over their free ends (Fig. 1, *h*). It was thus seen that the wall of the gas-bubble is composed of the mucus-like secretion of the basal disc. The basal ectoderm is, therefore, concerned with the elaboration of both the mucus-like material with which the hydra fixes itself to some submerged surface, and with the elaboration of a gas.

Our chief point of interest, however, lies not in the ectoderm of the basal disc but in the endoderm; for here we find a feature that has not been described. The endodermal cells of the basal region are larger than those of the middle and oral region, and bear great vacuoles. They do not ingest food particles (Fig 1, *e*). In this respect they again differ from the middle and oral regions' endodermal cells. Until recently these cells were supposed to overlie the basal disc's ectoderm Indeed Curtis and Guthrie ('27) have

illustrated these peculiar cells as lining the fundus of the enteron. In our gas-secreting specimens we find that the fundus of the enteron is lined with endodermal cells that bear numerous food-vacuoles. They are tall, columnar cells having myonemes in their bases. They resemble the epitheliomuscular cells of the middle region of the body. We have not been able to isolate them for maceration methods and cannot, therefore, say that they bear flagella. But they so closely resemble the epitheliomuscular cells of the middle region that our inference is that they do bear flagella. The presence of these cells, therefore, in the fundus of the enteron gives us an epithelial region that is like that of the oral and middle regions of the body except that there are no gland-cells in this basal endoderm.

The new histological region that we have thus discovered lies in the wall of the basal disc. The ectoderm of this region we have found to conform to the descriptions of earlier investigations; but the endoderm is peculiar in that it is an epithelial disc composed of columnar epitheliomuscular cells, that contain food-vacuoles, and which presents no gland-cells.

The presence of this endodermal disc, that is active in food-appropriation, is of interest when we keep in mind the dual function of the basal disc. The elaboration of gas takes place relatively rapidly. The rapid elaboration of gas would, therefore, involve rapid metabolism. The basal disc, then, is a region of relatively high metabolism. The presence of this food-getting endoderm in this region falls in line with the observation of Tannreuther ('09) when he says that "Those endodermal cells of the region of growth, 'the formation of buds and sexual organs', are the most active in ingesting partly digested food from the enteron and preparing it for diffusion into the ectodermal cells" (p. 211).

The basal disc has, therefore, a peculiar endoderm correlated with the dual function of mucus- and gas-elaboration.

The elaboration of gas is done in order that the specimen may be lifted in the water. The gas is discharged into the sac of mucus until the bubble formed lifts the animal to the surface of the water. As the polyp rises it has its basal end directed up and oral end pending. When the bubble encounters the surface film of water, its wall ruptures and forms a somewhat circular disc of mucus,

that is closely applied to the surface film of the water to form a raft by which the polyp hangs (Fig. 2, *g*). Wave-action will destroy this raft and then the specimen will sink. Or the hydra may abstrict itself from the raft of mucus and sink.

Summary.

We have observed that the basal disc's ectoderm in hydra not only secretes adhesive material, with which the polyp fixes itself to some submerged surface, but that it also under certain conditions elaborates a gas. This gas is caught within the mucus-like secretion of the basal disc and retained therein. The bubble, thus retained, increases in size until the hydra is lifted to the surface by it. At the surface of the water the retaining vesicle of mucus breaks and spreads as a circular raft from which the hydra hangs beneath the water's surface.

The ectoderm of the basal disc is thus seen to have a double function. Associated with this region of the ectoderm of hydra there has been found a pecular region of endoderm. The endoderm of this region is characterized by its component cells having the appearance of the epithelio-muscular cells of the oral two thirds of the body as over against the highly vacuolated epitheliomuscular cells of the aboral third of the body.

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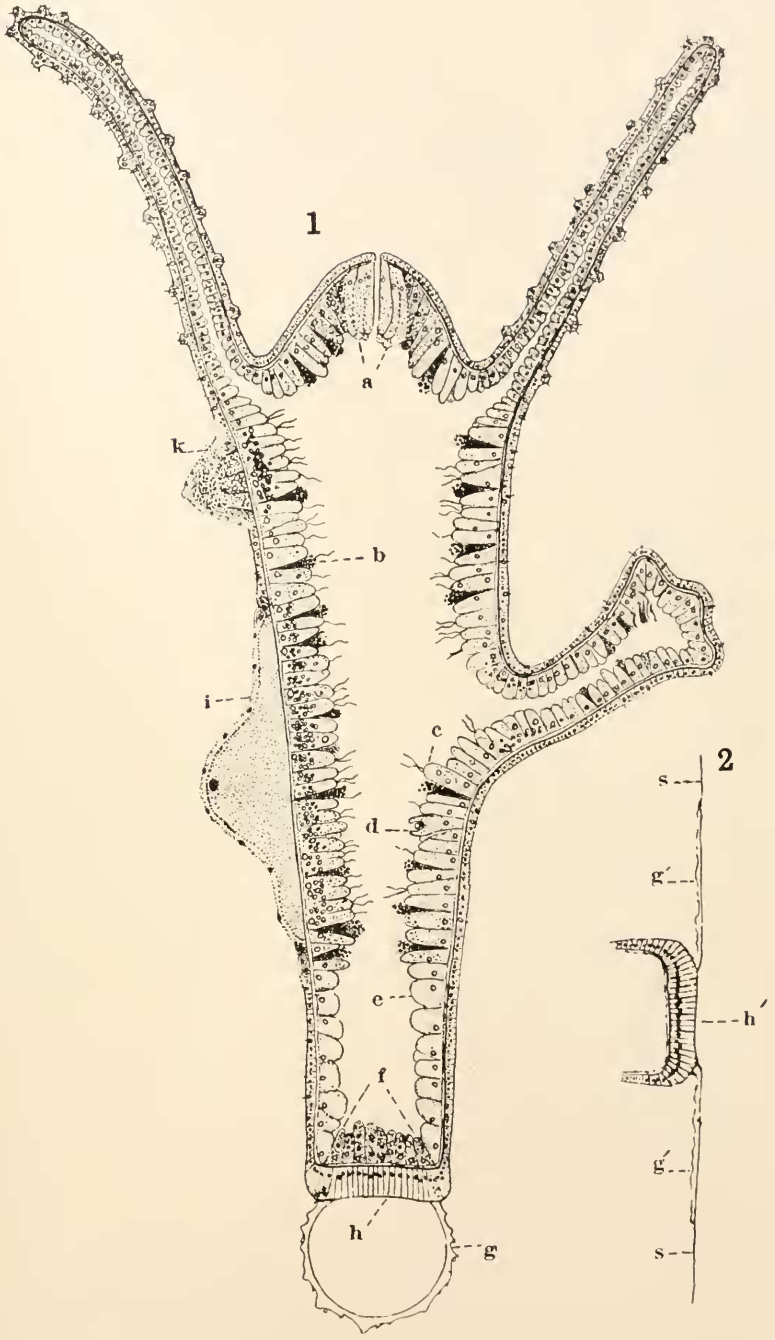
EXPLANATION OF PLATE.

FIG. 1. Longitudinal, axial section of hydra. *a*, peristomal gland-cells of endoderm; *b*, gland-cell of middle region's endoderm; *c*, epitheliomuscular cell of endoderm, bearing two flagella and two food-vacuoles; *d*, epitheliomuscular cell of endoderm ingesting a food particle; *e*, epitheliomuscular cells of lateral endoderm of basal region; *f*, endoderm of basal disc; *g*, mucus-wall of the gas bubble; *h*, basal disc's ectoderm that is exposed to lumen of gas bubble; *i*, ovary; *k*, testes.

FIG. 2. Basal disc of polyp at surface film of water. *s*, surface film of water; *g'g'*, circular raft formed by ruptured wall of gas bubble now applied to surface-film of water; *h'*, ectoderm of basal disc that is now in contact with air.



WM. A. KEPNER AND LULU MILLER.

HISTOLOGICAL FEATURES CORRELATED WITH GAS SECRETION IN *HYDRA OLIGACTIS* PALLAS.

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The ectoderm of the basal disc of hydra ordinarily elaborates mucus by means of which the polyp adheres to some submerged surface. It has recently been observed that this same ectoderm may, under certain circumstances, elaborate gas. This gas is held within a sack of mucus (Kepner and Miller, '28). This fact has suggested to us that there may be some interesting histological change correlated with the elaboration of gas. Kepner and Miller have found, to begin with, that there is a peculiar region in hydra's endoderm in the basal disc. This endoderm supplies the food demanded by the metabolism that yields the two products of the basal disc's ectoderm—mucus and gas.

Their observation concerning this local accumulation of food was interesting; for it carried the point, arising out of Tannreuther's ('09) observation, a step further. Tannreuther's observation was that in the region of a developing gonad or of an incipient bud there were local and pronounced accumulations of food within the endoderm. Yoder ('26) found that there was a marked accumulation of glycogen in these regions of most active metabolism. Finally Kepner and Hopkins ('24) observed that there was not a wide diffusion of material absorbed by the endoderm. For example, they recorded that "there is no extensive diffusion of absorbed chloretone through the tissues of the body. A diploblastic animal, therefore, cannot possess anything comparable to a circulatory medium" (p. 448). Our observations further strengthen the hypothesis that local needs must be met locally.

The detailed histology of the basal third of a hydra, that had not been secreting gas, shows the lateral endodermal cells to be stout and highly vacuolated (Fig. 1, *c*). The extent of distribution of these lateral cells varies greatly in different specimens and

perhaps also in the same specimen at different phases of the polyp's activity. The cells of the endoderm of the basal disc are more slender and present a denser cytoplasm than do the cells of the above lateral endoderm. They also carry within their cytoplasm food vacuoles (Fig. 1, *f*). Food-vacuoles are not present in the lateral endoderm of the basal part of hydra. The lateral ectoderm of the basal third does not in any manner differ from that of the general ectoderm of the body. The ectoderm of the basal disc, however, presents features that are peculiar to it. In the first place, there are no nematocysts in this region, of the outer epithelium. These cells, moreover, when actively discharging mucus have conspicuous inclusions within their cytoplasmic bodies. These may be designated secretion-granules (Fig. 1, *m g*). These

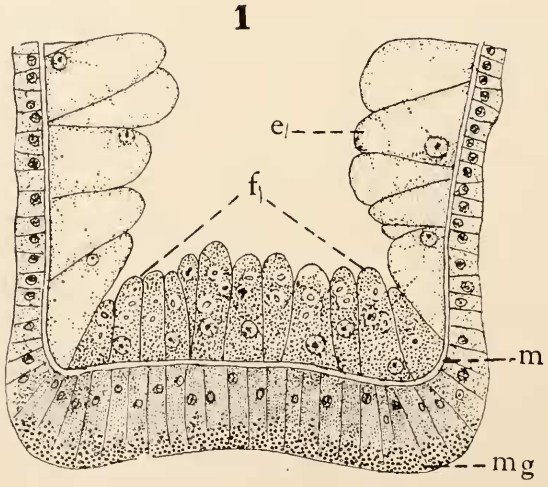

FIG. 1. Partly diagrammatic, axial section of base of hydra that had been elaborating mucus in order to fix itself to substratum. *e*, lateral endoderm of basal third of body; *f*, peculiar endoderm that lines the fundus of the enteron and contains food-vacuoles; *m*, mesoglea; *mg*, mucus-granules arising within the mucus-secreting cells of ectoderm. × 650.

cells extend over the entire basal disc. All attached hydras will not show the secretion-granules in the ectodermal cells throughout the extent of the basal disc. A specimen that has long been attached to the substratum may not yield, when fixed and stained, sections that display secretion-granules. Only specimens caught at the time they are fixing themselves to a substratum will present

these secretion-granules in sections (Fig. 1, *m g*). The most emphasis must here be placed upon the condition of the mesoglea. In a mucus-secreting specimen, the mesoglea of the basal disc differs in no manner from that of the body proper. It presents a uniform, unbroken contour (Fig. 1, *m*). All this in no manner presents anything new concerning the histology of hydra.

In the gas-secreting hydra some conspicuous histological features appear that have not been recorded. In the first place, the endodermal cells of the basal disc appear to be larger and more active in the gas-secreting specimens than in the non-secreting ones. Next we find that the ectodermal cells of a wide peripheral region of the disc elaborate mucus. These, in other words, do not have their usual function changed. The axial cells in the basal disc's ectoderm, however, do have their function altered. They no longer present secretion-granules and therefore stain (in hæmatoxylin) less than do the peripheral cells of the disc. We have now an epithelium the periphery of which elaborates a retaining wall of mucus, while the axial region secretes gas into the mucus to form a buoy or lifting float for the polyp (Fig. 2, *mc* and *gc*). The most conspicuous feature of the gas-secreting polyp lies in the mesoglea's modification. The mesoglea, in this disc at this time, becomes greatly swollen and highly vacuolated as though the endoderm had flooded it with a deposit of metabolic substance. Within this broken region of the mesoglea there appears, in fixed material, a substance that suggests a coagulated plasma (Fig. 2, *m'*). The presence of this plasma within the mesoglea may signify one of two things: (1) It may be that food is being deposited there by the endoderm in order to meet the demands of an intense metabolism taking place during gas-elaboration, or (2) It may be that metabolic substances are being dammed back from the relatively active axial ectoderm while it is elaborating gas. If the first alternative be correct, a plasma, as it were, is thrown down locally into the axial mesoglea of the basal disc in order that the gas-secreting cells may be abundantly supplied with food during the peculiar metabolism involved. If the second alternative be correct, it means that the axial ectoderm is throwing metabolic wastes into the mesoglea. Ordinarily the ectoderm discharges its metabolic wastes externally through a moist mucus when the

polyp is fixed, or directly into the water when the polyp lies free. When, however, a layer of gas is deposited upon the outside of this basal, axial ectoderm the osmotic drainage is blocked. The presence of the gas no longer lets the metabolic wastes drain by means of an osmotic exchange through the free ends of the axial, ectodermal cells. Hence metabolic wastes, instead of metabolic food, back into the mesoglea to flood it and form the vesicle that we have observed. As the elaboration of gas advances the vesicle of the mesoglea, together with its included plasma, decreases until, at the time of the gas-buoy's attaining its maximum size, the mesoglea has returned to the condition characteristic of the general mesoglea.

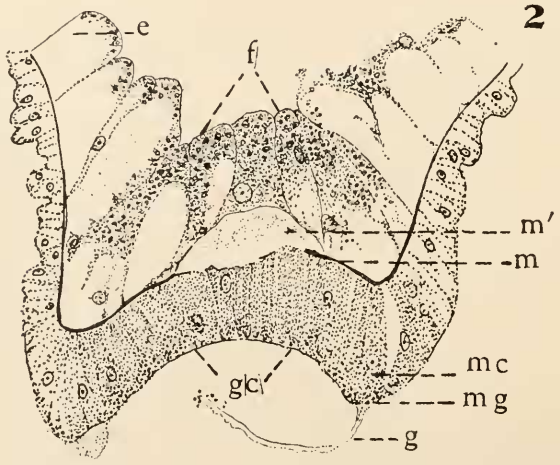

FIG. 2. Axial section of base of hydra that had been elaborating a gas-bubble. *e*, lateral endoderm of basal third of body; *f*, peculiar endoderm that lines fundus of enteron and contains food-vacuoles; *gc*, gas-secreting region of ectoderm; *mg*, mucus-granules; *g*, mucus wall of gas-bubble; *mc*, mucus-secreting region of ectoderm; *m* unbroken, but deeply staining region of mesoglea; *m′*, plasma-like material within distended region of basal mesoglea. × 650.

It matters not which of the above alternatives be the correct interpretation, the interesting point may be made that, in the basal disc of hydra secreting gas, we have a situation arising that makes a peculiar demand upon the passive mesoglea. As a result, the mesoglea is sometimes flooded either with metabolic food or with metabolic wastes. Thus we have the mesoglea foreshadowing the

function of a true circulatory medium such as is found in the plasma of the Turbellaria or even in that of the blood and lymph of the higher triploblastic animals.

Summary.

The basal disc of hydra has a two-fold secretory function: (1) It secretes a mucus by means of which the polyp is anchored. (2) It secretes gas that is retained within a wall of mucus by means of which the polyp is lifted to the surface of the water. This dual function of the basal disc places a peculiar metabolic demand upon the disc's endoderm, ectoderm and mesoglea. As a result of this peculiar metabolism and the conditions arising out of gas-secretion, the mesoglea becomes flooded with a plasma and thus handles either metabolic food or metabolic wastes in a manner that foreshadows the plasma of the circulatory media of triploblastic animals.

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THE LIGHT-RECEPTIVE ORGANS OF CERTAIN BARNACLES.[1]

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CONTENTS.

Introduction.

Although brief references to the light-perceptive organs of barnacles are found throughout the literature, no really comprehensive study has been made of them since that by Pouchet and Jobert in 1876. The relationships between the "eyes" in the larval stages and those in the adult have never been fully worked out. Accordingly the object of this investigation has been a study of the various light-perceptive organs found in the larval and adult barnacles and the determination of their relationships.

Investigations relating to the light-receptors of barnacles are usually found combined either with studies of other structures of the barnacle or with studies of the eyes of Crustacea in general. Among the former type Darwin's "Monograph of the Cirripedia" (1851 and 1854) is outstanding. More recently Gruvel (1905) has published a detailed account of the morphology and taxonomy of this group. Grenacher (1879), Claus (1891), Brooks and Herrick (1892), and Demoll (1917) have studied the eyes of Crustacea. General problems in this connection have been taken up by Parson (1831), Patten (1886 and 1887), and Mark (1887).

[1] The author is indebted to Dr. J. P. Visscher of Western Reserve University for the suggestion of the problem and for assistance during the course of the investigation.

Materials and Methods.

The ivory barnacle, *Balanus eburneus,* and the rock barnacle, Balanus balanoides, were used in this study. Collections were made at Woods Hole, Mass., during the spring and summer of 1926. Living forms were observed at this time and material was preserved for subsequent study.

Adults of *Balanus eburneus* brought to the laboratory often contained Nauplei nearly ready for hatching. Unhatched Nauplei were obtained by removing the ovigerous lamellæ from these adults, and free swimming forms were collected after hatching. A few metanauplei and Cyprids were obtained from towings made in the vicinity.

In order to test the actual light-perceptive function of the adult, the shell was broken so that the eyes could be exposed. Specimens with the shell thus broken were tested to make sure that the light reaction was unimpaired. The eyes were then removed by a hot needle. In all cases included in the data, the eye adhered to the needle and was removed without apparent injury to the surrounding tissue.

Material was fixed in picro-acetic formol (Bouin's) or in 10 per cent. formalin, and was either stained in Ehrlich's hematoxylin or mounted unstained. Most of the study was done by means of sections which were prepared by the ordinary paraffin method and cut from 5μ to 10μ in thickness. Grenacher's technique for the removal of pigment (Lee, 1890) was used on sections of the Cyprids. In addition a modification of Cajal's silver nitrate technique as developed by Hess (1925) was used in the study of the adult eye.

The Nauplian Stage.

The formation of the median eye in Cirripedes has never been worked out. In *Balanus eburneus* the eye first appears in the unhatched Nauplius as an elongated area of reddish pigment. Certain authors, for example Darwin (1851), have believed that the nauplian eye might arise from the union of two anlagen but, at least in *Balanus eburneus,* this does not appear to be the case. A large number of specimens in which the eye was just forming were studied but in all cases it appeared as a single area with no

evidence of a double origin. In succeeding stages the pigment becomes darker and the eye appears as a bilobed structure.

The morphology of the nauplian eye of Cirripedes has usually been treated incidentally in connection with studies of the complete animal and consequently its detailed structure is known in only a few forms, chiefly among the Lepadæ. The pigmented area in the anterior region of the Nauplius was noticed by the earliest authors but their descriptions mention only the general shape of the pigment cup. A fairly complete study of the light receptors in the Cirripedia was made by Pouchet and Jobert in 1876. These authors described the nauplian eye as formed of two parts each of which represented a simple eye and was composed of a pigmented body with finely granular, rose-colored pigment and small oval bodies which stained black with osmic acid and

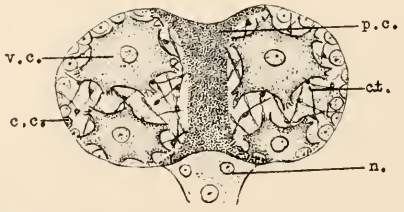

Fig. 1. Diagrammatic drawing of the median eye in the cyprid stage (longitudinal vertical section × 500). *c.c.*, cortical cells; *c.t.*, connective tissue; *n.*, nerve; *p.c.*, pigmented cells; *v.c.* visual cells.

which they thought might be designated as lenses, although the analogy was somewhat questionable. The median eye of Crustacea in general was described by Demoll (1917) as an inverted eye composed of two pigment cells forming a cup in which were the visual cells which extended proximally in a nerve fibre. These visual cells were in the nature of "Stifchensäume" and the whole eye cup was lined with fine, reflecting scales which formed the "tapetum." A cellular lens was said to be present in some forms.

When sections of fully developed Nauplei are studied the median eye (Fig. 1) can be seen lying between the so-called "cerebral ganglia." The eye at this period measures about 15μ in diameter and, due to this small size, an interpretation of its structure is very difficult. The central region is composed of

heavily pigmented cells which form a bi-concave pigmented cup. In each concavity there is a non-pigmented area coated by cells with large nuclei, similar to ones which form the cortical area of the cerebral ganglia. The non-pigmented area appears to be made up of two visual cells surrounded by a small amount of connective tissue. Each area sends off a nerve which is probably formed by an extension of these visual cells as in the adult. These nerves leave the posterior region of the eye and by very short connectives enter that part of the ganglia which is slightly posterior to the eye.

A lens as described by Claparède (1863) for *Lepas* is not present in *Balanus*. The non-pigmented area composed of visual cells and connective tissue was probably mistaken for a lens by Pouchet and Jobert (1876). This description is not in accord with that given for the median eye of other Crustacea by such authors as Hesse (1901), and Demoll (1917). The eyes of the two Cirripedes studied show ganglion-like visual cells instead of the " Stabchen " or " Stiftchensäume " described by these authors. While it is difficult to determine accurately the morphology of the median eye at this period, the structure, as described, agrees fully with that observed later in development. Hanström (1927) in a recent article has described a somewhat similar structure in the larval eye of *Nymphon stroui*. Whether the lack of agreement concerning the structure is due to error on the part of the foregoing authors or whether there is a real difference in the median eye of these Cirripedia is a point which has not yet been determined.

In addition to the median eye characteristic of the Nauplius, the Metanauplius of *Balanus eburneus* has a pair of compound eyes which are generally known as the cyprid eyes since they are the most noticeable light-perceptive organs of that stage. The cyprid eyes in the Metanauplius appear on either side of the median eye near the base of each of the first pair of nauplian appendages. Their structure at this period is superficially the same as in the Cyprid but material was not available for histological verification of the appearance. The nauplian eye retains its characteristic position and appearance during the metanauplian stage.

In common with the Nauplei of other barnacles those of *Balanus*

eburneus show definite reactions toward light. In a dish containing Nauplei these were ordinarily found congregated in the area having the greatest illumination. Occasionally a few individuals were observed which collected at a point just opposite this brightest spot. The reason for this variation has not been determined but it seems to be constant in such specimens and therefore is not the same type of reaction as that described by Groom and Loeb (1891) in which the same individuals responded differently under different conditions.

The Cyprid Stage.

The early authors had not observed the Metanauplius stage of Cirripedia and so did not always clearly recognize the distinction between the median and compound eyes. Burmeister (1834) described the median eye as a rounded, black spot which became divided and modified to form the paired, compound eyes of the Cyprid. This error persisted through several of the later works and Darwin (1854) reported Burmeister's observation although he considered it " scarcely possible that the eye of the larva of the first stage can be changed into the double eyes of the second stage." It was not until the two types of eyes were observed present at the same time that this misapprehension was fully removed.

The fate of the compound eyes at metamorphosis into the adult has been variously described. Darwin (1851) and Hesse (1874) noted that the eyes fell from their capsules during metamorphosis while Von Willemoes-Suhm (1876) stated that before metamorphosis the eyes lost their original position and might be seen only as black pigment spots which were later absorbed. A similar observation has been made by Coar [1] in an unpublished study of *Balanus balanoides* and by Hanström (1927). The author has observed the extruded eyes in slides of *Balanus amphitrite* collected by Dr. J. P. Visscher at Beaufort, North Carolina. However, the condition in *Balanus eburneus* seems to be similar to that described by Von Willemoes-Suhm and Coar. The two late cyprid stages which were observed at metamorphosis showed no compound eyes, the region of these being occupied by pigment masses which may have been the degenerating eyes.

The compound eye, as seen in total mounts, has been described

[1] Personal correspondence, 1926.

by all authors studying the Cyprid. In such preparations the eye appeared as consisting of a black pigment body and eight to ten globules or lenses surrounded by a large capsule, and is described as such by Darwin (1851), Hesse (1874), and Von Willemoes-Suhm (1876). When studied in section the compound eyes of *Balanus balanoides* (Fig. 2) are found to be situated in pockets near the bases of the antennules. In contrast to the rather unique structure of the median eye, their appearance is very similar to that of the compound eyes of other Crustacea. The eight to ten lenses described for similar forms by early authors are present and represent the same number of visual elements or ommatidia. Each ommatidium contains a cuticular lens surrounded by "corneagen cells," which are reported by Patten (1886 and 1887) to

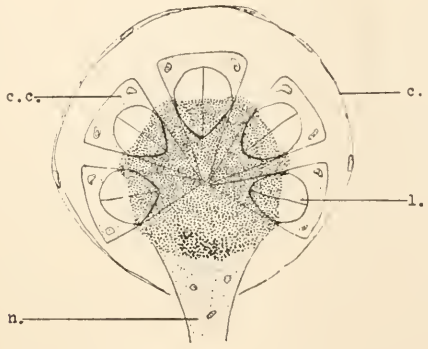

FIG. 2. Diagrammatic drawing of the compound eye in the cyprid stage (vertical section × 300). *c.*, cornea; *c.c.* corneagen cells; *l.*, lens; *n.*, nerve.

secrete this body, and other cells known as the cells of the crystalline body. The lens or crystalline body is made up of three units forming an egg-shaped structure. The proximal portion of the ommatidium contains the rhabdomes surrounded by heavily pigmented retinular cells. This region is much shorter in the compound eyes of Balanus than in most Crustacea. It was not possible to study the rhabdome in detail but it appears to be like that of other Crustacea, of reticular nature and penetrated by nerve fibrils. The retinular cells pass into the optic nerve of the com-

pound eye. The ommatidia are surrounded by a common corneal sheath. The structure of the compound eye of *Balanus eburneus* appears to be similar, as far as could be determined from a study of total mounts.

In 1851, Bate noted that the pigmented area of the Nauplius was represented by a similar region in the Cyprid although he did not believe that the area was a light-receptor in either period. Claus (1869), in a drawing of an unknown Cyprid, pictured the persistence of the unpaired eye up to the moment of metamorphosis, although the eye is not labelled and it is probable that he did not realize the significance of his observation. Pouchet and Jobert (1876) recognized and discussed this persistence of the median eye through the cyprid stage.

The median, or nauplian, eye (Fig. 1) persists throughout the cyprid period. It has enlarged to five times its former diameter (15μ–79μ) but its structure is the same. The cells with large nuclei still coat the non-pigmented areas and because of the similarity of these nuclei to those of the regular visual cells, it is extremely difficult to determine the exact number of the latter. The bi-concave pigmented area is composed of many cells in contrast to the condition reported for *Lepas,* where only two cells are found. In each pigmented area there are two non-pigmented zones each containing two visual cells which form a nerve connection with the cerebral ganglia. These nerve connections have elongated while the amount of connective tissue surrounding the visual cells has also become greater and the reticular nature of part of it is evident in most sections.

It is difficult to say just what part the compound and median eyes play in the light perception of the Cyprid. Probably both are functional. Since the median eye is functional in the stages preceeding and following this period, as well as after the degeneration of the compound eyes, it is unlikely that it would completely lose its function at this time.

The Adult Stage.

Early observers denied the presence of an eye in adult barnacles and indeed the relatively degenerate structure and enclosing shell of the adult tended to support this view, as well as the fact

that all eye structures were thought to be lost at metamorphosis, with the disappearance of the cyprid eyes. The first report on the existence of eyes in the adult barnacle was made by Leidy (1848) on *Balanus rugosus* (sp?). In 1854, Darwin substantiated Leidy's report by finding eyes present also in the adult of *Balanus tintinnabulum*. A summary of previous studies of the barnacle eye was made by Gerstaecker (1866). These were concerned chiefly with the fact of occurrence or with external appearance of the eyes. The most complete study of the barnacle eye in the adult was that made by Pouchet and Jobert in 1876.

There seems to be no published report of the origin of the eyes in the adult. Darwin (1854) pointed out that they were not developed from the eyes of the Cyprid, since the new eyes were formed at some distance from the compound, but thought they might have been formed from the nauplian eye since they occupied a similar position. Coar [1] reported that in *Balanus balanoides* the adult eyes were formed by a division of the median or nauplian eye, and the author has found that the same situation occurs in *Balanus eburneus*. The eye of the Nauplius, which has persisted throughout the cyprid stage, divides into two parts during the metamorphosis of the Cyprid into the adult. These, together with a part of the mantle which becomes modified around them, form the simple, paired eyes of the adult. In animals which have just completed metamorphosis the two eyes may be seen completely separate although still very close together (Fig. 3 *A*). They move apart during the succeeding period and, at about the fifth day, are in the position which they occupy in the adult (Fig. 3 *B*). The eyes were found to lie in the mantle between the scutum and the juncture of the rostrum (rostrum coalesced with rostro-lateral-Darwin 1851) and the lateral plates of the shell. Immediately around the eye and optic nerve the mantle lacks its usual pigmentation and for this reason the eye appears prominent. The part of the eye toward the body of the barnacle is heavily pigmented while the region toward the shell is without pigment.

Pouchet and Jobert (1876) described the eye as a rounded structure partly covered by pigment and adherent to the surrounding tissue "en arrière." They believed that this pigment func-

[1] Personal correspondence, 1926.

tioned as a choroid coat while the non-pigmented area might possibly be called a cornea. When the eye was macerated in Müller's fluid they noticed the presence of a cell which owing to its volume, granular nature, and distinct nucleolus, they felt was undoubtedly a nerve cell. The existence of a double optic nerve suggested to them the possibility that there were two such cells. However, they never observed more than one and were inclined to consider the situation analogous to that in the Lepadæ where they had found a double nerve but always a single nerve cell. The light was described as reaching the eye by traversing the tissue which united the valves and which contained no pigment in the vicinity of the eye. They found that barnacles were sensitive to light,

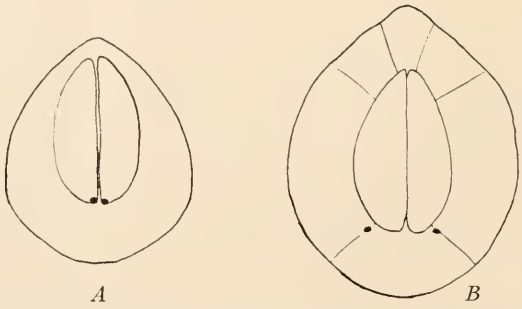

FIG. 3. Diagrammatic drawing showing adult of *Balanus eburneus*. *A*, immediately after metamorphosis; *B*, five days after metamorphosis.

but did not believe that they possessed object vision. No mention is made of the origin of the adult eye although development from the median eye is suggested by the nature of the remarks.

When sections of the eye of the adult are studied it is seen to be composed of two main divisions; an outer covering which is a modification of the mantle and an inner part which is developed from the divided nauplian eye (Fig. 4).

The outer covering is composed of irregularly shaped cells similar in appearance to those of the mantle. Pigmented cells make up about half the area of this coat and are continuous with like cells in the mantle, while non-pigmented cells form the rest of the covering and are a continuation of similar mantle cells.

Between this covering and the inner portion of the eye is a region of loose collagenous connective tissue fibers which serve

to hold the inner region in position as well as to support the outer covering. In the living specimen the interstices of these fibers are filled with fluid which helps to maintain the contour of the eye.

The inner part of the eye is a sphere containing pigmented cells, collagenous fibers, reticular fibers, and visual cells with their nerves. The pigmented region forms a cap over about half of this portion of the eye and lies directly beneath the pigmented region of the outer coat. Beneath this, is an area of rather loose connective tissue fibers and cells similar to those between the outer and inner regions. The rest of the inner eye is composed of a reticulum of connective tissue fibres surrounding two large ganglion or visual cells. A slight division is observable in this

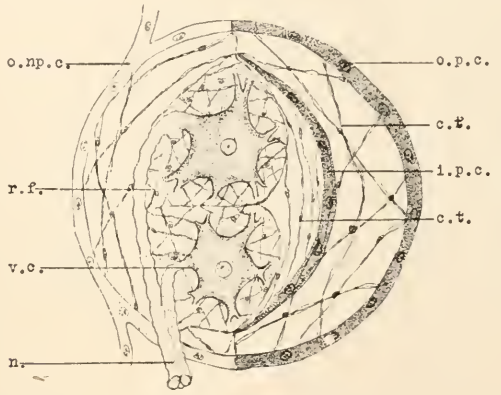

FIG. 4. Diagrammatic drawing of the simple eye of the adult (vertical section × 20). *c.t.*, connective tissue fibres; *i.p.c.*, inner pigmented cells; *n.*, nerve; *o.np.c.*, outer non-pigmented cells; *o.p.c.*, outer pigmented cells; *r.f.*, reticular fibers; *v.c.* visual cells.

reticulum, indicating the two units of nervous elements. These two visual cells send off branches which ramify throughout the reticular fibers. Each cell also gives off a nerve fiber which is surrounded by a sheath. These fibers, with their individual sheaths, become united as a double nerve enclosed within a common connective tissue coat before passing through the outer covering and continuing to the supraœsophageal ganglion as the optic nerve.

The enlargement of the nerve just prior to its entrance into

the pigment mass, as described by Gruvel (1905), is not apparent in the sections and it seems probable that he has included the non-pigmented region of the outer coat as a part of the nerve. Since his work did not include studies of sections of the eye, this error is very natural.

Papers on the median eye of crustacea often describe the presence of a tapetum next to the two (or more) cells which form the eye cups. By homology the inner pigment region of the adult barnacle eye corresponds to these cells and the tapetum might therefore be either the loose connective tissue or the reticular regions of this eye. However so little uniformity is found in the use of this term by investigators that it is considered inadvisable to apply the name to any particular region of the barnacle eye.

Barnacles in their natural environment will retract their cirri when stimulated by light, and this fact is mentioned by several of the early authors. Pickering (1848) brought it forward in confirmation of Leidy's report as to the existence of eyes in the adult. Darwin (1854) observed the reaction to light in *Balanus balanoides, Balanus crenatus,* and *Chthamalus stellatus* and found that they were all sensitive to a shadow produced by passing his hand between them and the light. Gerstaecher (1866) reported experiments by Fr. Müller who found that *Balanus tintinnabulum* would react to a shadow when the body was removed and the eyes and certain muscles were left in the shell. ("dass *Balanus tintinnabulum* auf eine Beschattung mit der hand auch dann reagire, wenn er mit Zurücklassung seiner Augen an dem Manteldeckel, von diesem abgelöst werde. Ein in dieser Weise entblösstes, mit halb entrollten Ranken im Wasser liegendes Exemplar zog dieselben jedesmal schnell ein, wenn es beschattet wurde.")

In our study it was found that the adults of both *Balanus balanoides* and *Balanus eburneus* close the opercular valves if there is a sudden change in light intensity although very gradual changes do not excite the reaction. *Balanus eburneus* is more sensitive to such changes than is *Balanus balanoides.* A slight shadow may sometimes be cast upon the latter without affecting them but it was never possible to do this with *Balanus eburneus.*

Since it is shown that the adults possess some light-perceptive mechanism, it becomes necessary to find what part the so-called

"eye" plays in causing this reaction. Accordingly, the light reactions were studied in twenty-five adults of *Balanus eburneus* which had been deprived of these organs and it was observed that such animals showed no reaction to light changes, however sudden or intense, although when the eyes were intact they had all withdrawn their cirri and closed the valves under similar conditions. Therefore it is concluded that these organs are the sole light-perceptors present in the adult barnacle.

The function of certain parts of the eye is problematical. Since the ganglion or visual cells send branches throughout the reticulum of connective tissue fibers immediately surrounding them, it is evident that this part serves in the transmission of the impulse. The looser collagenous fibers outside this area do not have any self-evident function. As they are not pigmented they would not prevent the passage of light and it seems probable that their function is merely that of support. The inner pigmented cells may be either protective or reflective in nature. The structure of the eye and its inverted nature lend some support to the latter possibility. The light enters the eye through the non-pigmented area of the outer coat while its entrance through the other cells is prevented by the pigment. The fluid which is found in the eye of living specimens must act as a refractive as well as a supporting medium.

Summary.

1. The development, structure, and function of the light-perceptive organs are described in the nauplian, cyprid, and adult stages of *Balanus eburneus* and *Balanus balanoides*.

2. The light-perceptive organs present in the various stages are: (*a*) nauplian—a median eye, (*b*) metanauplian—a median eye and two compound eyes, (*c*) cyprid—a median eye and two compound eyes, (*d*) adult—two simple eyes.

3. The median eye in *Balanus eburneus* originates as a single, pigmented mass in the unhatched Nauplius and persists with no change, except in size, until the metamorphosis of the Cyprid into the adult.

4. The compound eyes first appear in the metanauplian stage and remain functional throughout the cyprid stage.

5. These compound eyes are resorbed at the time of the metamorphosis of the Cyprid into the adult.

6. At the metamorphosis into the adult the median eye divides into two parts which form the simple paired eyes of the adult.

7. Each of the paired eyes in the adult is the morphological equivalent of half of the median eye plus an outer covering.

8. The simple, paired eyes are the sole light-perceptive organs of the adult.

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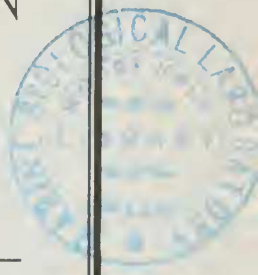

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